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**INTRODUCTION**  
**TO**  
**ELEMENTARY PRACTICAL**  
**BIOLOGY**

**A Laboratory Guide**  
**for**  
**High-School and College Students**

**BY**  
**CHARLES WRIGHT DODGE, M.S.**  
**PROFESSOR OF BIOLOGY IN THE UNIVERSITY OF ROCHESTER**



**NEW YORK**  
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*" Study nature, not books."*—AGASSIZ

*" Real knowledge in science means a personal acquaintance with the facts, be they few or many."*—HUXLEY

*" The ideal of scientific teaching is, no doubt, a system by which the scholar sees every fact for himself, and the teacher supplies only the explanations."*—HUXLEY



## PREFACE

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STUDENTS, particularly those of the natural sciences, are no longer satisfied to receive didactic instruction, but want the training which will fit them for investigation. In Biology it is the custom to furnish such training by having the student verify the facts acquired by professional investigators. The methods of teaching now in vogue for elementary classes are methods of *instruction* rather than of *education*. The student gets the most of his knowledge from books or from lectures, and very little from specimens. When thrown upon his own resources, as when called upon to examine some natural object and to describe it in his own language, he is entirely powerless. If he can find in some book a description of the object, he may, perhaps, be able to verify it, and, using that in the book as a pattern, to write a description of his own.

A method of teaching which consists solely of the verification of the printed statements often leads students into temptation, for there are always a few who have too little mental strength and moral courage to resist using as their own the facts given in books, particularly when it comes to dissecting a difficult organ or to tracing a minute duct. Not a few students who

have been taught by the "verification method" have confessed committing to memory the detailed descriptions of their specimens in preference to doing the work necessitated by a practical examination. It is needless to say that with such students laboratory work fails completely to accomplish its fourfold object—viz., to teach the student, first, to observe correctly; second, to distinguish essential from non-essential facts; third, to draw proper conclusions from the facts observed; and fourth, to express in writing and by means of drawings the results obtained.

For the purpose of developing in the student the power of independent observation, to teach him that the source of all knowledge is not "the book" rather than the specimen, that there is a great deal to be seen besides that which "the book" describes, and that he is not to consider that, though his observations may not agree with those of "the book," the latter must, necessarily, be right—these are the reasons why this guide has been written.

The guide consists essentially of questions on the gross and minute structure and on the physiology of a series of common animals and plants which are typical of their kind—questions which can be answered only by actual examination of the specimen or by performance of the experiment. Directions are given for the collection of specimens, for their preservation, and for preparing them for examination, also for performing simple physiological experiments. Particular species are not required, as the questions usually apply equally well to several related forms.

The material of which the guide is composed has been gradually accumulated during an experience of nearly seven years of teaching high-school and college classes, consisting of students of both sexes and of ages varying from fourteen to fifty years. With the exception of a few additions made while preparing the manual for printing, all of the work herein detailed has been performed by college students, and a very large part by students in the second year of their high-school course.

Although it is thought that the topics are so arranged and the questions so worded that any one of average intelligence can study any or all of the organisms given, still it is not intended that the guide shall supersede the instructor, but, rather, aid him. The student will always require the advice and suggestions of his teacher, who will need to point out many of the structures which here are only named, and which it would be impossible to describe so that the student could find them for himself, without defeating the very object which the guide seeks to attain.

As this manual has to do entirely with the work of the laboratory, it is suggested to those teachers who wish to use a text-book also, that nothing better can be found than Parker's admirable work, "Lessons in Elementary Biology," 2d ed., New York, 1893.

Following the classification given in the "Text-Book of Zoölogy" of Claus and Sedgwick, and the "Outlines of Classification and of Special Morphology of Plants" of Goebel, an attempt has been made to arrange in logical sequence the organisms to be studied, proceeding from the simple to the more complicated. It is desira-



ble that they should be studied in this order. Experience has shown that this method can be pursued with little or no difficulty and to the greatest advantage. It is very frequently the case, as here, that the simplest form is not necessarily the best known. Every one is more familiar—by sight, at least—with the frog than with the amoeba. The structure of the former resembles that of the human body far more than does that of the latter. But how many students have even the most general knowledge of human anatomy? They know, to be sure, that the body contains a heart, lungs, stomach, etc., but in the great majority of cases would fail to locate or, if shown them, even to recognize these organs. Again, how many students, if called upon to do so, could tell more about the frog than that it usually lives in the water, is greenish in color, has four legs, a mouth, etc., and can jump and swim? Whether or not the frog has a tail is usually a question for discussion. As a matter of fact, beginning students have no more real knowledge of the higher than of the lower forms.

That the student is unaccustomed to the use of the microscope is often urged as a reason against beginning with minute organisms. Does his study of the gross anatomy of a rabbit or of a frog teach him anything about the manipulation of this instrument? Sooner or later he will have to learn to use it, and it matters little when he does so; the instrument will be unfamiliar to him until he actually handles it and learns its use by degrees. He should approach it by way of the magnifying glass, and use the low before attempting the high powers. In two hours the average student can learn

sufficient of manipulation to warrant trusting him with a microscope. His power of observation will be developed only by training. It is advisable to begin with the lower forms, not only because of their simplicity of structure, but also because of the value of this method in tracing the development both morphologically and physiologically of the tissues and organs of the higher organisms and the evolution of forms. Though this plan seems preferable, it is not absolutely necessary that it should be followed. The student may begin with the highest form and work downward; or he may commence with an intermediate form and work either way; again, he may begin with an intermediate form, then go to the lowest and work upward. On account of lack of instruments, it may be necessary to omit all of the minute organisms and all of the microscopic work on the higher forms. It is thought that the guide can readily be adapted to any of these methods. The end to be attained is not to examine as *many* specimens as possible, but to examine them as *thoroughly* as possible.

It has been found that the question stimulates the student to a degree of mental endeavor far beyond that attained by the attempt to verify a printed statement. A few of the questions are suggestive rather than capable of a definite answer, but these will be found to have their use. The notes made in the laboratory should form the basis of the recitation, and many of the topics should be further elaborated and discussed by the teacher.

Much of the pleasure and instruction to be derived

from the examination of living organisms is lost because few people know how and what to observe. To most individuals the study of Natural History means nothing more than the memorizing of Latin names, of long descriptions consisting of unfamiliar terms, of "collecting" various kinds of disagreeable creatures, and preserving them dried or in alcohol, etc., etc.

Should this guide, even in the slightest degree, prove instrumental in doing away with such a belief and in cultivating a taste for the study of nature, then will it have accomplished its purpose.

In one of the appendices is given, under its appropriate heading, a list of literature which the student may profitably consult *after* having finished the study of each organism. In the construction of the guide free use has been made of the works named. In another appendix is given a brief list of the reagents and some of the appliances needed in carrying out the work given in the body of the manual. For more detailed directions and descriptions the student is referred to the works on microscopical technique and laboratory methods.

Acknowledgment is due to Mr. Arthur Willey, of Columbia College, who kindly looked over the proof-sheets and made a number of valuable suggestions.

C. W. D.

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## INTRODUCTION

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EVERY student should have assigned him a definite place to work, for whose good order he should be held responsible; also a drawer in which to keep his instruments, note-books, etc. The drawer ought to be provided with a lock to which no one but the student and the instructor has the key. The most convenient place for the drawer is in the table at which the student sits, as here it is more easily accessible than elsewhere. If necessary, a case of drawers may be placed at some convenient place in the room.

The laboratory exercises should be at least two consecutive hours in length, as so much time is consumed in making the preparations for work and in "cleaning up" afterwards that but little can be accomplished in one hour, while an exercise which lasts three hours is very likely to fatigue the beginning student unduly.

A certain fee ought to be charged for the use of microscopes and other apparatus, and for specimens, etc. Some wear and tear of instruments is to be expected, but any damage beyond this should be made good by the student.

Economy of material must be practised, but not to such an extent as to interfere with obtaining a clear idea of the structure of each organism. Each student should provide his own specimens of the commoner forms, e. g., earthworm, locust, and various plants. Before putting specimens into hardening reagents, be sure to see that the body and its appendages are properly arranged, otherwise they may stiffen in positions awkward for handling. Preserved specimens

of the marine forms may be obtained from the Department of Laboratory Supply of the Marine Biological Laboratory at Wood's Holl, Mass., or from Ward's Natural History Establishment, Rochester, N. Y.

Whenever possible, the living organism ought to be closely observed before any work is done on dead specimens. It is an excellent plan for the student to have a live specimen before him while studying the anatomy of dead material.

### **Apparatus required by the Student**

Each student should provide himself with the following :

1. A medium-size and a small scalpel.
2. A pair of medium-size and a pair of small (oculist's) forceps, both with straight points and rough tips.
3. A pair of fine curved scissors, which should meet accurately at the tips.
4. A pair of dissecting needles, made by fastening the eye-end of stout sewing-needles into wooden penholders.
5. A razor.
6. Twenty-five slides and a half-ounce of cover-glasses.
7. Two note-books, one for making condensed notes while doing the laboratory work, the other in which to write in *ink* a full and *carefully worded* account of the observations made.
8. A number of cards of bristol-board cut to the size of a large postal-card. Upon these drawings are to be made. If desired, a blank-book of thick calendered paper may be used.
9. A hard pencil, either HHHHHH or HHHHHH Faber is recommended.
10. A piece of india-rubber.
11. An apron, preferably of rubber.
12. A towel.

### **Dissection**

The object of dissection is to separate the various parts in such a manner as to display their shape and mutual rela-

tions, and it consists almost entirely in the removal of the connective tissues which bind the different organs together. The student must plainly understand that hacking specimens to pieces is *not* dissection.

While dissecting, the following rules should be borne in mind :

1. Fix the specimen in whatever position may be most convenient for work. If it be a large object, as, for example, a lobster or frog, lay it dorsal or ventral side uppermost as may be desired, and with the head turned away from one. If the specimen be so easily moved as to interfere with the work, stick pins obliquely through the softer tissues into the dissecting-tray or the wax in the bottom of the dissecting-pan.

2. The tissues of fresh specimens must be moistened from time to time with water or with normal salt-solution to prevent the drying and distortion of parts. Alcoholic material should be examined in dishes containing fifty per cent. alcohol. If such specimens be large, e. g., lobster, and it be desirable to examine them on the dissecting-tray, place them for two or three hours previous to the time when wanted in a mixture of equal parts of water, alcohol, and glycerine. They will then keep moist even in the open air.

3. Before making a cut in any direction, study the specimen carefully, and note where the cut must be made in order to expose the part wanted with the least injury to surrounding parts. Specimens unnecessarily mutilated should be replaced at the expense of the student.

4. In dissecting and cleaning muscles, nerves, and blood-vessels, stretch them slightly and work in the direction of their length, *never* crosswise.

5. Avoid unnecessary handling of the parts, and do not pinch them with the forceps.

6. Do not allow scraps to accumulate on the specimen. Sponge away blood-clots. With a pipette wash away the debris which accumulates on specimens dissected under water, or change the water frequently.

7. Good work cannot be done with dull instruments, therefore keep them clean and sharp.

8. When work is finished for the day, all instruments which have been used should be washed, or wiped with a damp cloth, to remove all adhering scraps of flesh, thoroughly dried with a soft cloth, then carefully oiled or rubbed with vaseline. The joints of the scissors should receive close attention.

It is a good plan to require each student to prepare a careful dissection of some animal or system of organs. These preparations may be used to form a laboratory museum.

### **Taking Notes**

A complete and carefully prepared description must be made of every specimen examined. This description will consist mainly of answers to the questions given in the manual. To these are to be added whatever independent observations the student may make. If the answers be given in the same order as the questions are asked, it will be found that the former constitute a brief essay upon the organism examined.

Two note-books should be used, one in which to write hastily in the laboratory the results of the observations made; the other to contain the carefully prepared descriptions which, written plainly in ink and with due regard to rhetorical form and expression, are intended for examination by the instructor and for permanent preservation. Make the laboratory notes full and complete, record the observation as soon as it is made, leave nothing to the memory; otherwise the second set of notes will suffer. In answering such questions as are mainly theoretical, give all the reasons you can *pro* and *con*.

### **Drawing**

Draw every specimen examined and every dissection made. Endeavor to get correct outlines and relative posi-

tion of parts. Shading is seldom desirable. Draw everything to scale, and mark on the drawing the scale adopted. Always sketch in the outline faintly at first, then fill in the details. When the specimen is bilaterally symmetrical, draw a faint line to represent the median line, sketch the outline of the left, then of the right side, and fill in the details. Make every drawing large enough to show all of the parts plainly. Name every part or organ in every drawing. It adds much to the appearance and value of the drawings if they be colored with water-colors, using the dull tints for the larger and the bright colors for the small organs. In a set of drawings of the same specimen always use one color for the same system or organ. Indicate arteries in red, veins in blue, and other parts in their natural colors.

*Drawings must not be made too small.*

### Using the Microscope

1. Take the instrument from the case together with the eye-piece and objective to be used, then close the case and set it aside out of the way.

2. Examine every part of the instrument to be sure that it is clean. Wipe off the dust by brushing lightly with a *soft clean cloth*.

3. If there be anything but dust on the objective, wipe the latter with a soft, moist cloth, then dry immediately with a soft cloth. If this fail to remove the dirt, take the objective to the instructor. Do not use chamois skin or soiled cloths of any kind. A worn linen or silk handkerchief, kept perfectly clean and free from dust, is as good as anything that can be found to clean the microscope.

4. Put in the eye-piece ; screw on the objective.

5. Incline the tube at a convenient angle.

6. Turn the tube down to about one fourth of an inch from the stage. *Be exceedingly careful to avoid contact of the front lens with the stage or with anything upon it.*

7. Arrange the mirror so as to throw the light *which comes in at the window* up through the opening in the middle of the stage. Do not use the direct sunlight.

8. Place the glass slide on the stage and under the clips, with the object over the centre of the opening. If the object be mounted in a fluid, see that none of the latter oozes out around the edge of the cover-glass, otherwise the objective may be soiled. Never use the high power to examine an object which is not protected by a cover-glass.

9. Turn the tube down *nearly* to the object.

10. While looking through the microscope, bring the object into focus by slowly turning the tube *upward*. *Never* do so by turning the tube downward.

11. Examine every object with the *low power* first; keep both eyes open when examining an object. This can be done after a little practice, and avoids unduly fatiguing the muscles which close the unused eye. Get into the habit of using either eye. With the low power use a large and with a high power a small diaphragm. Use the concave mirror for strong illumination. If foreign particles appear in the field, locate them by the following method: While looking into the instrument rotate the eye-piece in the tube; if the particles also rotate, they are on or in the eye-piece and should be removed; if the particles do not rotate, move the slide; if the particles move, they are on the slide and must be wiped off. If the object looks hazy, the particles must be on the objective, which may be gently wiped; if this does not remove the difficulty, take the instrument to the instructor. While observing, vary the focus constantly with the fine adjustment. This method will give a far clearer idea of the object than to fix the focus once for all and to sit staring into the tube.

12. On quitting work, turn the tube up to about an inch above the stage, remove the slide, unscrew the objective and put it into its case, remove the eye-piece and return it to the box, straighten the instrument if it has been in-

clined, and place it in the case, being careful to lock the latter.

13. *Never* touch the lenses or the mirror or the surface of the slide or cover-glass with the fingers. Wipe off dust with a soft cloth. Hold slides and cover-glasses by the edges.





**PART I**

**THE BIOLOGY OF THE CELL**



I  
THE ANIMAL CELL,  
ITS MORPHOLOGY AND PHYSIOLOGY

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A.—THE UNICELLULAR ANIMALS

EXAMPLE 1.—The Protozoa

*Material.*—Specimens may be obtained in any of the following ways: by skimming the surface of the mud in the bottom of a pond or a slow-running ditch or brook; by scraping the under surface of the leaves of water plants, as pond-lilies, duck-weed, etc.; by squeezing the water out of clumps of fresh-water or marine algæ, or out of damp sphagnum moss; by tying a small bag of bolting-cloth or of fine muslin over the mouth of a water-tap, allowing the water to run slowly through the bag for fifteen minutes to an hour, then turning the bag wrong side out and rinsing the collected sediment into a dish containing a small amount of water; by searching, under the microscope, the moist green film found growing on the surface of flower-pots, trunks of trees, and on the bricks or stones in the foundations of buildings; by examining the fluid found in the mantle-cavity of oysters and clams; by rinsing in artificial sea-water the gills of unboiled lobsters; by scraping the tongue and the roof of the mouth of frogs

or ducks, or the gills of fishes a day or two after they are caught; by rinsing the contents of the intestines of frogs, lobsters, crawfish, etc. Many marine forms may be caught by skimming the surface of the water with a net of bolting-cloth, especially on warm, quiet evenings. The most practicable method of supplying specimens for large classes is to resort to cultivations. Fresh-water forms may be raised in large numbers by cutting into a dish bits of hay, grass, marsh-grass, potato, fish-skin, moss, or, in fact, almost any organic substance, pouring over the pieces just enough lukewarm water to cover them, and setting the dish, covered to prevent evaporation, in a warm, not too light place for one to seven days. Another very satisfactory method is to allow fresh-water weeds and algæ to decay in a small amount of water. Many marine forms may be cultivated by soaking in artificial sea-water for a few days bits of oysters, clams, and the gills of unboiled lobsters. As particular forms usually have definite habitats, it is best not to obtain all of the specimens from a single locality, but to gather them from as various sources as possible. In the cultivations some forms will be found at the edge of the dish at the surface, others on the bottom, others attached to the sides, some swimming freely, and still others fastened to the pieces of organic material.

To study the specimens properly the student will need the following apparatus: A compound microscope magnifying from fifty to four hundred diameters, slides and cover-glasses, and a pipette.

*Method of Examination.*—Take a clean glass slide, with the pipette put in the centre of the slide a drop of water thought to contain specimens, lay a piece of hair

or a scrap of thin paper or a bit of wax by the edge of the drop, then carefully place the cover-glass on the drop, resting one edge upon the hair or paper so that the cover may not crush the specimens by its weight. Care must be taken to keep the specimens supplied with water. This can be done by placing a drop at the edge of the cover-glass from time to time. Capillary attraction will draw the drop between the slide and the cover. Examine the preparation first with a low, then with a high power. Be careful not to mistake for Protozoa various microscopic worms and crustaceans, usually distinguishable by their appendages.

*General Questions.*—How many different shapes can you distinguish? What variations in size? In color? Is the body always symmetrical? What determines the shape of the body? Is there any distinction between *head* and *body*? What various motions have these animals? By means of what organs are the movements produced? Do all of the Protozoa which you find possess the same kind of motor organ? In what manner are the motor organs connected to the body? Does the body contain *blood*? Can you find any organ corresponding to a *heart*? *Stomach*? *Lung* or *gills*? *Brain*? How do these animals eat? Digest their food? Breathe? Are *eyes* present? How do the animals find their way about? Do they *feel*? Are *nerves* visible? How do the Protozoa know what to eat? Is their soft body, consisting mainly of *protoplasm*, protected in any way? What means of offence or of defence have these animals? Means of distribution? How do you account for their very wide distribution? For the possibility of raising them artificially in the ways described above?

Make sketches of six of the different forms which you find.

**EXAMPLE 2.—The Proteus Animalcule (*Amæba Sp.*)**

*Material.*—Good specimens may usually be found by scraping the slimy surfaces of water plants, washing damp sphagnum moss, skimming the mud in the bottom of ponds and ditches; or raised artificially by keeping such mud, together with decaying leaves, algæ, and other water plants, in a warm, dark place for a few days. Such cultivations should be closely watched, as *Amæbæ* are likely to disappear rapidly in the course of a few days after they are first found. A little fresh water should be added to the cultivations from day to day. If *Amæbæ* are to be kept in aquaria, be careful to see that all snails are removed, otherwise they may devour the specimens.

The apparatus needed is the same as given for the general study of Protozoa, with the following additions: camera-lucida, scale for measuring, dilute iodine, one per cent. acetic acid, one per cent. acetic acid carmine, or fuchsin, powdered indigo or carmine, bristles, warm-stage, alcohol lamp, glass rod, egg, and gum-arabic.

*Method of Examination.*—The same as for Protozoa. It is very likely that several drops of water may have to be examined before specimens are found. In searching for them, use the low power ( $\frac{3}{8}$ -inch or  $\frac{1}{2}$ -inch objective); then study with the higher power. Take up a little of the sediment along with the drop of water to be examined. Specimens of *Amæba* may be kept in good condition for several days in succession by placing them in the moist chamber.

Having found some good specimens, study carefully the following:

## MORPHOLOGY

- a. Size.*—Is *Amœba* visible to the unaided eye? Compare several as regards size. Make a camera drawing of an average specimen and find its exact size, using the scale prepared for the microscope.
- b. Shape.*—What is the shape? What determines it? Is it constant? Why? Note the projections, or **pseudopodia**, of the body which are thrust out at intervals. How many may a single animal have? How few? What is the shape of a **pseudopodium**? Is this shape invariable? Do the pseudopodia ever branch? Do different specimens vary in the number and size of their pseudopodia? What significance in the common name of the animal? Make six sketches of the outline of the body at intervals of two minutes each.
- c. Structure.*—Distinguish the following parts:
1. The **ectosarc** (outer, clear layer of protoplasm).—How much of the body does it form proportionately? Is it visible over the entire body? Where is it most plainly seen? Is there any skin or membrane outside this layer?
  2. The **endosarc** (inner, granular mass of protoplasm).—How much of the body does it form? Is it distinctly separated from the ectosarc? Compare with the latter as regards consistency. Does the endosarc ever appear on the outside of the body mass? Are all the granules of the same size? What shapes and colors have they? Are they stationary? Why? What other things besides granules are visible in the endosarc?



3. The **nucleus**.—What is its position? Shape? Size? Is it visible in each specimen? Is it always seen in the same place when visible at all? If not plainly to be seen apply a drop of dilute iodine, of one per cent. acetic acid, or of one per cent. acetic acid carmine to one edge of the cover-glass, and allow the stain to run under the cover. Watch the effect on the other parts of the animal also. If the stain be used, fresh specimens must be prepared before going on with the study of the contractile vacuole. Do you find more than one nucleus? Is there a nucleolus? In stained specimens the nucleus will appear darker than the surrounding protoplasm.

4. The **contractile vacuole**.—Position? Shape? Are position and shape constant in the same and in different specimens? Does the vacuole have any visible contents? Do you find specimens having more than one contractile vacuole? If there are several vacuoles, are they all contractile?

Make a drawing to illustrate the structure of *Amœba*.

#### PHYSIOLOGY

a. *Movements*.—What kind of motion does *Amœba* exhibit? Is the motion continuous? Regular? Does the body change shape when moving? What part of the body is used to produce movement? Watch the manner in which a pseudopodium is formed. Is it always from the same part of the body? What part does the ectosarc play in the process of moving? Endosarc? Does the endosarc extend into the pseudopodium? Study

the movements or *pulsations* of the contractile vacuole. How frequent are they? Are they regular? By what do they seem to be produced? What becomes of the contents of the vacuole during a contraction? Do the contractions affect the granules in the endosarc? What do you take to be the function of the contractile vacuole? Is it possible that it serves more than one function?

b. *Nutrition*.—What does the animal eat? How does it obtain its food? How is the food taken into the body? Grind a small piece of indigo or of carmine in water, mix a drop with that on the slide, and watch to see *Amæba* ingest the particles. Note, also, the movement of these particles around through the body mass. Where is the mouth? In what part of the body is the food digested? Why does not *Amæba* digest itself? How is the digested food distributed to different parts of the body? Where does the waste matter leave the body? In what manner does the animal breathe? What breathing organs does it have? Where are they situated?

c. *Sensation*.—If the specimens be large the cover-glass may be carefully removed, or a fresh specimen prepared without the cover, and, while examining with a low power, the animal may be carefully touched with the point of a fine bristle. Does *Amæba* give any indication that it feels such an irritation? Tap the slide with a pencil. How does the animal behave? Put a covered preparation on the warm stage, and heat slowly to about 45° C. What visible changes take place in the animal? With another covered specimen

heat a small glass rod in the flame of an alcohol lamp, and touch the heated rod to the cover-glass in the neighborhood of the animal. What result?

*d. Reproduction.*—Look for specimens in the process of *fission* or *division*. If one be found, note how the process takes place and the length of time required. Compare the two resulting bodies as to size and structure. Which is the parent? Is the process preceded by sexual union? How is one sex distinguished from the other?

*General Questions.*—How does *Amæba* protect itself from its enemies? Kill an *Amæba* by crushing it under the cover-glass. What changes take place in the protoplasm? Why does not the protoplasm of a living *Amæba* go through the same changes? Do the granules of a crushed *Amæba* continue to move? How does this movement (if any) compare with that observed in the living *Amæba*? Compare with the crushed and with the living *Amæba* a drop of the white of egg and a small piece of gum-arabic, both in water. What differences can you detect? How do you explain them? Does *Amæba* have any *organs*? Why?

**EXAMPLE 3.—The Slipper Animalcule (*Paramecium Sp.*)**

*Material.*—Specimens may almost always be found in water which contains a considerable amount of decaying vegetable matter. They are usually abundant around the decaying stalks and leaves of pond-lilies. They may be raised in enormous number in the course of a week or two by placing in a warm, dark place a fruit-can containing water in which are dead fresh-water algæ or other decaying vegetable substance.

Use the same apparatus as for *Amœba*, with one per cent. solution of chloral hydrate and one per cent. solution of osmic acid additional, also a saucer and a piece of pasteboard.

*Method of Examination.*—The same as for *Amœba*. *Paramecium* is an exceedingly active animal except when feeding, hence it is well to have a supply of decaying vegetable tissue in the drop which is being examined. Specimens may be brought to rest by allowing the water between the slide and the cover-glass slowly to evaporate, or they may be caught in the meshes of a thin layer of cotton-wool which may be spread out on the slide. As the cover settles down the animal will be caught and held fast. Care must be taken not to allow the cover to crush the specimen. This may be avoided by adding from time to time at the edge of the cover small drops of water just sufficient to make up for the loss by evaporation. Or, a drop of one per cent. solution of chloral hydrate or of one per cent. acetic acid may be added to the drop containing the specimen; either of these reagents, however, will kill the specimens in the course of a few minutes. They may be killed in condition satisfactory for examination by placing the slide with the drop of water downward over the mouth of a bottle containing a one per cent. solution of osmic acid. Exposure to the fumes of this acid usually causes the death of *Paramecium* in two to five minutes.

It is best to examine the active animals before attempting to use the reagents.

#### MORPHOLOGY

a. *Shape.*—What is the shape when the animal is at rest? Does it change during locomotion? Com-

pare with *Amæba*. What determines the shape? Is there any variation in shape as the animal assumes different positions? Is the body symmetrical? Has the animal an anterior and a posterior end? How are they distinguished? What is the significance of the animal's common name? Make drawings to show the shape of the animal as seen in different positions.

b. *Size*.—How does *Paramecium* compare in size with *Amæba*? Examine several specimens to see if there are any variations in size. Is the animal large enough to be seen without a microscope? Compare length with breadth. Make a camera drawing of a specimen and find its actual size.

c. *Structure*.

1. The **cuticle** or **cell-wall**.—Note its extreme transparency and flexibility. Is it present over the entire surface? Is it a complete film—*i. e.*, is it entirely without openings? Place some specimens in a drop of water on a slide, but do not put on the cover-glass. Note the appearance of the cuticle as the water evaporates and the body of the animal becomes dry. Can you discover any variations in thickness? Any markings on the surface?
2. The **ectosarc**.—Is it similar to that of *Amæba*? Account for any differences that you may find.
3. The **endosarc**.—Compare with that of *Amæba*. Look carefully for food-vacuoles—*i. e.*, drops of fluid containing particles of ingested substances.
4. The **contractile vacuoles**.—How many do you find? Where are they situated? Examine sev-

eral specimens to see if the vacuoles are constant in number and position. What is their shape when expanded? When contracted? Are they in the ectosarc or endosarc?

5. The **nucleus** (better called the **macronucleus**).  
—What position has it? Shape? Structure? If the nucleus be not easily seen, treat the specimen with stains as directed for *Amœba*. After staining look for a small body, the **paranucleus** (better called the **micronucleus**), lying by the side of the nucleus. How does the nucleus of this animal compare in shape and size with that of *Amœba*?
6. The **cilia**.—Are they found covering all parts of the body? How do you prove this or the opposite? Do they have any definite arrangement? Are there any variations in size? Is there any connection between the ectosarc and the protoplasm composing a single **cilium**? Examine the form, structure, length, and diameter of a single cilium. This may best be done in animals killed with one per cent. osmic acid or one per cent. acetic acid, or on specimens stained with iodine. Considering form and size, how many kinds of cilia can you distinguish? Make enlarged drawings of the various kinds.
7. The **trichocysts** (seen lying directly beneath the cuticle).—How are they arranged? What is their shape? Structure?
8. The **mouth**.—What is its position? Shape? Is the position constant? Is the shape permanent? How is the mouth closed? Compare with *Amœba* in these various respects. Why does *Paramecium* have a mouth?

9. The **œsophagus** or **gullet**.—What is its shape? How far does it extend? How do you distinguish its lower end? Look for cilia lining the gullet.
10. The **anus** (seen during the ejection of waste matter).—Where is it? Is its position permanent?
11. The **food-vacuoles**.—In what part of the body are they seen? What is their shape? Of what are they composed? Are all of the same size? Compare several animals to find the average number contained.

Make drawings showing the relative position, shape, and size of all the parts studied.

#### PHYSIOLOGY

##### a. *Movements.*

1. *Of the entire body.*—What different motions has the body? What are the organs of motion? Are all the movements of the body produced by these organs? If not, how are the exceptional movements produced?
2. *Of the cilia.*—What sort of motion have the cilia? Is it the same for all? What causes their motion? Are all of the cilia used for the same purpose? Considering *use* alone, how many kinds of cilia are there?
3. *Of the trichocysts.*—Run a drop of one per cent. acetic acid or of dilute iodine under the cover-glass. What happens to the trichocysts? From their behavior when the animal is thus irritated, what do you judge their function to be?
4. *Of the contractile vacuoles.*—What sort of motion have they? Does each go through the same

movements? What causes their motions? Do they contract at the same time? Expand? What becomes of their contents during contraction? How long is the time between two consecutive contractions of the same vacuole? Is the time uniform? Compare the rate of contraction or *systole* with that of expansion or *diastole*. Are other parts of the body affected by the movements of these vacuoles? The motions of the contractile vacuoles are often seen to advantage by allowing the water under the cover-glass to evaporate, thus causing the slow death of the animal.

5. *Of the food-vacuoles*.—What kind of motion is it? To what is it due? Is it regular? Do all of the food-vacuoles move? Do all move in the same direction? At the same rate? Are they affected by the movements of the contractile vacuoles? Of what use is the motion?

*b. Nutrition.*

1. *Food and method of feeding*.—Judging from the contents of the body, what does the slipper animal eat? By staining specimens with dilute iodine, which colors starchy substances blue and albuminous substances yellowish-brown, it will be possible to determine the nature of some of the ingested substances which cannot be recognized by their shape. Watch the ingestion of food-particles. How does it take place? Is it always through the mouth? Is there any other way for food to enter the body? Compare with *Amœba* in this respect. How is the food swallowed? Study the formation of a food-vacuole.



How does it take place? How often is such a vacuole formed when the animal is feeding quietly? What changes take place in a food-vacuole during its passage around the body? How is the refuse matter passed out of the body? What causes the changes noticed in the particles of food in the body? The slipper animalcule may be fed with particles of indigo or of carmine in the same manner as described for *Amæba*.

c. *Nervous Properties.*

1. *Automaticity.*—What determines the motions which the animal makes, the food which it selects, the times for resting or moving, etc., etc.? Does it appear to perform actions “of its own accord”? Is it possible to predict what the animal’s behavior is to be at any given moment? In what part of the body does this property seem to reside?
2. *Irritability.*—Does the animal appear to feel objects with which it comes in contact? Does it respond in any way, as by movements of various kinds, to external stimuli?
3. *Co-ordination.*—Do the cilia move rhythmically, or does each move independently of all the others? What regulates their motions? Do their motions seem to be made for any purpose?
4. *Special Senses.*—Do any of your observations lead you to think that the slipper animal has the sense of touch? Does it exercise selection in the choice of its food—*i. e.*, has it the sense of taste? Place a shallow dish—*e. g.*, a saucer—painted black inside and then coated with

shellac, containing a large number of slipper animals, near a window; agitate the water in the dish so as to distribute the animals evenly. Cover half of the dish with some opaque object as a book or a piece of pasteboard, in order to make one side of the dish darker than the other. Let the dish stand quietly during four or five hours of daylight. At the end of that time examine the water carefully with a hand-lens to see whether the animalcules have collected more abundantly on the dark or on the light side. Can the slipper animal see?

*d. Reproduction.*

1. *Fission*.—How does this take place? How does the process begin? How long does it last? How many bodies result from it? Which is the “parent” and which the “child”? What becomes of the “ancestor”? Is the process common? How do the resulting bodies compare in shape, size, and structure with the original? Can you detect, with or without reagents, that any changes take place in the nucleus and paranucleus?
2. *Conjugation*.—How many individuals take part in the process? How long does it last? What portions of the body are in contact? How many bodies result from the process? Is the process voluntary? How are two individuals in process of conjugation to be distinguished from one individual in process of fission? Is the conjugation permanent or do the individuals separate again? How are the sexes distinguished? Is any choice shown by those

engaged in the process? What changes are noticed in the animals after conjugation?

Make drawings showing the process of fission in several different stages and of conjugation.

*General Questions.*—Which is the “higher” animal, *Paramecium* or *Amœba*? Why? What various means of dispersal has *Paramecium*?

**EXAMPLE 4.—The Bell Animalcule (*Vorticella* Sp.)**

*Material.*—Specimens of *Vorticella* may usually be found in the water which contains *Amœba* and *Paramecium*. Fine examples are frequently found attached to the stems and leaves of aquatic plants and to the filaments of algæ, also to shells and stones.

Use the same apparatus, reagents, and stains as for the slipper animal.

*Method of Examination.*—The same as given for the slipper animal.

Use in general the questions and directions given for the study of *Paramecium*, with, however, the following additions, the omitted numbers indicating corresponding parts of the two animals.

Under *Shape* insert:—Look for colonies of *Vorticella*. Are the individuals connected in any way? Compare the shape of the body and stalk in the expanded state with the shape of the same parts in the contracted condition. What is the shape of the body when seen from above? From the side? Is the common name appropriate? Why?

Under *Structure* insert :—

- 1'. The **stalk**.—What is its shape? Structure? With what parts of the body is it connected? Examine the axis of the stalk. Of what is it composed? Look for *striations* in its substance. Is the axis attached to the cuticle of the stalk? If so, in what manner? Compare the diameter of the stalk with its length. How much longer is the stalk when expanded than when contracted? Does the stalk correspond to any part of *Paramecium*?
- 2'. The **peristome** or the rim of the body.—What is its position? Shape? How is it formed? Is it complete?
- 3'. The **disk** (lying within the peristome).—Position? Shape? Structure?
7. The **myophan layer** or striated base of the body.—Is it formed of cuticle, ectosarc, or endosarc? Are the striations constant? Does this correspond to any part of *Paramecium*?
- 7'. The **vestibule** or entrance to the gullet.—Position? How formed? Has the slipper animal any part corresponding to this?

Make a drawing showing all of the parts visible when the animal is expanded; another showing all that can be seen when the animal is contracted.

Under *Movements* insert :—

1. Of what use is the stalk? Study the manner in which the stalk contracts. What part of the stalk causes the contraction? How do you tell? What changes take place in the body during contraction? Note the rapidity of the movements. How does the animal assume the

expanded condition? What causes it? What part is the first to resume the expanded state? Compare the rate of contraction with that of expansion. Note that the body sometimes separates from the stalk. How does the body move after separation? What becomes of it? What position does the stalk assume? What reasons can you give for such behavior of body and stalk?

2. *Ciliary movement*.—What differences between the bell animal and the slipper animal in this respect? What use does *Vorticella* make of its cilia? What is the significance of the animal's scientific name?

Under *Reproduction* insert:—

3. *Encystation*.—Look for bell animals which have become encysted. How do they differ from the others? Of what use is this process? What seems to be the cause of it?

Make drawings of specimens undergoing fission; conjugating; encysted.

*General Questions*.—To what different uses do you find in general the various parts of the bell animal's body adapted; in other words, to what extent do you find the principle of the "physiological division of labor" carried out in this simple animal? From your study of the *Protozoa*, as exemplified by *Amoeba*, *Paramecium*, and *Vorticella*, what do you consider to be some of their principal characteristics and differences?

## B.—METAZOA OR MULTICELLULAR ANIMALS.

The following examples are cells from these animals, isolated from the body.

### EXAMPLE 5.—Salivary Cells

*Material.*—Rinse out the mouth two or three times with water, then collect some fresh saliva by spitting into a test-tube or a watch-glass. Let the glass stand quietly until the air-bubbles rise to the surface of the fluid and a sediment settles. The warm-stage, pipette, dilute iodine, magenta, one per cent. acetic acid, one per cent. acetic acid carmine, and indigo or carmine will be needed during the examination.

*Method of Examination.*—With the pipette put a drop of the sediment on the slide and lay the cover-glass in position, with one edge resting on a piece of hair or a scrap of paper. Examine first with the low, then with the high power. In the preparation will usually be found many colorless flat cells, with irregularly polygonal outlines and large nuclei. These are *epithelium cells* from the lining membrane of the mouth, etc., and have nothing to do with the proper salivary cells. It will be well to examine their shape, size, structure, and the manner in which they are connected. Note also the very large nucleus, which may be made more distinct by staining with magenta or acetic acid carmine.

### MORPHOLOGY

- a. *General Structure.*—What is the shape of the cell? Size? Color? Does it have a cell-wall? Nucleus? If so, what is its position? Shape? What does

the cell contain? Are the cells very abundant in the saliva? Do they in their structure resemble any of the *Protozoa*?

Run a drop of magenta under the cover-glass, and note the changes in the cells. Prepare another specimen, and treat with dilute iodine. Test a third preparation with one per cent. acetic acid; a fourth with one per cent. acetic acid carmine. In each case note the effect upon the entire cell and upon each of its parts. Of what use are these reagents and stains?

Make drawings illustrating the structure of the saliva cell.

#### PHYSIOLOGY

*a. Motion.*—Prepare another specimen as first directed, and place it upon the warm-stage, being very careful to keep the stage at the proper temperature—*i. e.*, that of the body.

Does the cell have the power of voluntary movement? Does it have cilia? Note the peculiar dancing motion or *Brownian movement* of the contained particles. Do you find any cells in the process of division?

*b. Nutrition.*—Grind a little indigo or carmine in water, and run a drop under the cover-glass. Watch the cells to see whether or not they ingest the particles. Note the Brownian movement of the particles of indigo or carmine. Do you think that this motion indicates that the particles are alive?

Make drawings illustrating any changes noticed in cells examined upon the warm-stage.

**EXAMPLE 6.—Blood Cells**

*Material.*—Chloroform a frog by placing it in a small box or under a bowl, with a piece of cloth or a small wad of cotton, upon either of which a few drops of chloroform have been poured. In four or five minutes the animal will be dead, though its muscles may twitch if stimulated. Such movements are purely reflex. Expose the heart by making an incision with a scalpel or with fine scissors along the median line through the skin and muscles of the abdomen, and turning back the flaps of skin and muscle. The heart will then be seen beating in the pericardium. Cut into this, and lay bare the tip of the heart. Have at hand, also, a hand-lens, compound microscope, one per cent. acetic acid, strong aqueous solution of magenta, warm-stage, indigo or carmine (dry), dilute yeast, piece of ice, alcohol lamp, .75 per cent. salt solution, pipette, and glass rod.

*Method of Examination.*—Put a drop of normal (.75 per cent.) salt solution in the centre of a slide, lay a scrap of paper by the side of the drop, snip off the tip of the frog's heart, collect a drop of blood in a pipette or on the end of a glass rod, and mix the drop with the salt solution. Put on the cover-glass and examine with a low, afterwards with a high power. Lay the frog away on a moist cloth or sponge under a bowl so as to prevent the body from becoming dry.

Note that the blood consists of a fluid or *plasma* in which float two kinds of cell—red and colorless.

**MORPHOLOGY**

**a. The red cells or red corpuscles.**—Are they abun-



dant or few in number? What is their shape as seen from the side? From the edge? What is the color? To what is due the color of the blood as a mass? Does the cell have a nucleus? If so, what is its position? Does it always have this position? What is the shape of the nucleus? Structure? Has the corpuscle a wall? Is the cell flexible or rigid? Does the red cell bear any resemblance to *Protozoa*?

Make drawings of red corpuscles as seen in different positions, showing both shape and structure.

**b. The colorless cells or "white" corpuscles.—**

What is their shape? Color? Size as compared with the red and with one another? Is there a cell-wall? A nucleus? How do these corpuscles compare in number with the red? Study this last point again by taking a drop of blood from the cut edge of a muscle half an hour after the incision was first made. Do you find any difference in the number of "white" corpuscles present? Compare the structure of one of these cells with that of *Amæba*. What resemblances and differences do you find?

Make drawings showing the shape and structure of "white" corpuscles.

Run a drop of one per cent. acetic acid under the cover-glass. How does the acid affect the internal parts of each kind of corpuscle? What changes are noticed in the color and shape of each? With a fresh preparation run a drop of a strong aqueous solution of magenta under the cover-glass, and note the effect. Run in a drop of distilled water in a fresh preparation. What becomes of the coloring matter of the red corpuscles?

Make a drawing of each kind of corpuscle after treatment with each reagent.

#### PHYSIOLOGY

Mix together on the slide a drop of frog's blood and a drop of normal salt solution, then examine with a high power.

*a. Movements.*—Do any of the corpuscles have the power of movement from place to place, or do any change their shape? If so, how is the movement effected? What sort of motion is it? Is it comparable to that of any animal which you have studied? Put the slide on the warm-stage and heat to the temperature of the human body. Does the higher temperature make any difference?

Make drawings to show the direction of motion and changes of shape if any.

To this drop of diluted blood add a drop of water in which indigo or carmine has been ground, or, better, use a drop of diluted yeast.

*b. Ingestion.*—Do any of the corpuscles ingest the particles of indigo or the yeast cells? If so, how do they do it? Compare with the ingestion of food-particles by the different kinds of *Protozoa* studied. Of what use could this property of the cell be in the frog?

*c. Effects of temperature.*

1. *Cold.*—Lay the slide on a piece of ice for five minutes, then examine, and compare the results with those of *a* and *b*.
2. *Heat.*—Heat the warm-stage slowly to a degree

uncomfortable to the touch, and note the changes taking place in the corpuscles.

Make drawings to illustrate the effect of the above experiments.

#### EXAMPLE 7.—Ciliated Cells

*Material.*—With a scalpel carefully scrape the roof of the mouth of a recently killed frog, and mount the scrapings on a slide in a drop of normal salt solution. Some single cells and some groups will be found. It may be necessary to use dilute iodine or one per cent. acetic acid carmine to demonstrate some of the structures. Ciliated cells may also be obtained by tearing into fine pieces the gills of a live clam.

#### MORPHOLOGY

- a. *General Structure.*—What is the shape of a single cell? How do you distinguish the upper end from the base? Compare several as to shape. Compare length with width. How are the cells arranged? What holds them together? Can you distinguish a cell-wall? Protoplasm? Nucleus? Contractile vacuole? On what part of the cell are the cilia?

Make drawings showing shape and structure of the cell.

#### PHYSIOLOGY

- a. *Movements.*—What part of the cell is capable of movement? Can the cell move as a whole? If so, by what means? What effect have heat and cold upon the movements?
- b. *Ingestion.*—Can the cell ingest particles of indigo?

*General Questions.*—In what respects does a ciliated cell resemble *Vorticella*? In what respects does the cell differ from the animal named?

From your study of the bodies of various *Protozoa* and of cells isolated from the bodies of higher animals, what can you give as some of the principal characteristics of animal cells? Do you think that these isolated cells may be considered as animals? Why?

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## II

### THE PLANT CELL, ITS MORPHOLOGY AND PHYSIOLOGY

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#### A.—THE UNICELLULAR PLANTS

##### EXAMPLE 1.—Yeast (*Saccharomyces Sp.*)

*Material.*—Dissolve a small piece of a “compressed” yeast cake in a little water, put a drop of the mixture on the slide, and examine according to the directions given for *Protozoa*. Or, a cake of dry yeast may be soaked in water until soft and a drop of the mixture taken for examination. If baker’s yeast is obtainable that may be used. In any case, disregard the starch grains which will probably be present. They may be recognized by being much larger than the yeast cells, usually oval in outline, and by having striations on their surface. Have at hand the magenta solution, five per cent. potash solution, dilute iodine, Schulze’s solution (dilute), a little corn-starch, a watch-glass, test-tubes, distilled water, ten per cent. sugar solution, Pasteur’s solution without sugar, Pasteur’s solution with sugar, Mayer’s pepsin solution, water-bath, barium hydrate, porous porcelain cup, litmus paper, moist chamber, two Florence flasks, chemical thermometer, teacup, alcohol lamp, dry plaster of Paris, camel’s-hair

brush, blotting-paper, absorbent cotton, U-shaped glass tube, baking-soda (bicarbonate of soda), and dilute muriatic or dilute sulphuric acid.

#### MORPHOLOGY

- a. Arrangement.*—How are the cells arranged? Do you find any single cells? Any groups? How many cells in a group? Are the cells arranged similarly in the various groups? Is this arrangement comparable to that of any *Protozoa* which you have seen? What holds the cells together? How many cells in a complete yeast plant?

Make drawings showing the various arrangements of the cells you find.

- b. Shape.*—What is it? Is it symmetrical? Are all of the cells shaped alike? Is the shape constant or does it change? Compare with *Amæba* and *Paramecium*.

Make drawings of single cells showing how they look in outline when seen from the side and from the end.

- c. Size.*—Is a single yeast cell visible to the unaided eye? With the scale measure the actual size of several cells. Do you find any variations in size? If so how do you account for them?
- d. Color.*—What is the color of a single cell? How does this compare with that of fluid yeast? Explain. Do you detect any green coloring matter or *chlorophyll* in the cells?

- e. Structure.*

1. The **cell-wall**.—Crush some of the cells by

gently tapping or pressing on the cover-glass, thus squeezing out their contents. What is the nature of its surface? What is its color? Is the color of the cell due to the cell-wall or to the cell contents? Do you find any variations in color and in thickness? Is there a mouth through which particles of food may enter the body? Can you detect any especial place for the absorption of fluids? Do you find any organs of motion? Is the wall at all elastic? How do you tell?

2. The **protoplasm**.—Does it entirely fill the cell? What is its color? Of what does it appear to consist? Examine some which has been squeezed out of the wall.
3. The **vacuole**.—In what part of the cell is it found? Do you ever find more than one? Is it contractile? Of what is it composed? How much of the cell does it occupy?
4. The **nucleus**.—Is the nucleus visible in every cell? What is its shape? Relative size? In what part of the cell is it usually found?

Make a drawing of a yeast cell, showing its structure in detail.

Run a drop of magenta solution under the cover-glass and note the effect on the cells. Which stain soonest and most deeply? What part of each cell becomes stained, and to what extent? On a fresh preparation try also one per cent. acetic acid carmine. With another preparation run under the cover-glass a drop of a dilute solution of Schulze's chlor-zinc iodide.

Treat another drop of yeast with dilute potash solution. What happens to the cells?

*f. Iodine test for detecting the presence of starch.*

1. Mix a little corn-starch or laundry starch in water in a watch-glass and add a drop of dilute iodine. What is the effect on the starch?
2. Put on a slide a drop of water containing starch and examine under the microscope. What is the color of the starch grains? Run a drop of dilute iodine under the cover-glass. What change takes place in the grains?
3. Mount a drop of yeast on a slide and treat with a drop of dilute iodine as above. What is the effect on the cells? Is starch present in the fluid yeast? Is there any starch in the cells themselves?

Make drawings of the yeast cells showing the effect of the reagents.

**PHYSIOLOGY**

In the following experiments the amount of growth which has taken place may roughly be measured by the increase of turbidity of the liquid in the test-tubes. It may be tested microscopically by the number of buds to which the cells give rise, by the amount of protoplasm, and the number of vacuoles in each cell. To begin with, the test-tubes must be as clean as possible.

- a. Effect of food-supply upon growth.*—Take five test-tubes each one-third full of the solution named: (1) distilled water; (2) ten per cent. solution of cane sugar in water; (3) Pasteur's solution without sugar; (4) Pasteur's solution with sugar; (5) Mayer's pepsin solution. With a glass rod put a drop of yeast into the fluid in each tube, being careful not to lose part of the drop by



touching it against the side of the tube, shake the tubes thoroughly, tightly plug the mouth of each with a wad of clean absorbent cotton, to prevent the entrance of dust, and set them in the water-bath, heated to  $35^{\circ}\text{C.}$ , for two or three days. Examine the tubes from time to time and notice what is taking place. More accurate results may be obtained in the following manner: Prepare five moist chambers, using a drop of each of the culture fluids given above, mix one drop of fluid yeast with about a thimbleful of distilled water, then mix a drop of this diluted yeast with the drop of culture fluid in the moist chamber, and watch under the microscope from day to day the development of the yeast cells in each kind of fluid. In which solution does the yeast grow best? In which solutions are bubbles of gas formed? Can you detect any relationship between the number of bubbles formed and the amount of growth? What relationship is there between the composition of the various solutions and the amount of growth which takes place in each? Is growth accompanied by anything else than the increasing turbidity of the solutions and the formation of bubbles? Do you find anything besides yeast cells growing in these solutions? If so, how do you distinguish them from yeast and how do you account for their presence? In what condition must the food be in order that it may be absorbed? Why does the cell absorb "food solutions"? Through what must the absorbed substances pass in entering the cell?

Taste of the ten per cent. sugar solution after

the yeast has been growing in it for a day or two. Compare with some in which there has been no yeast. What difference do you find? How do you account for it? Test the two solutions with blue litmus paper (which turns red when placed in an acid). What result? Explain. Make a thick syrup of sugar and water, put some into a test-tube, mix a drop of yeast with the syrup, and set the tube in the water-bath for a day. Does the syrup ferment? Can you explain why? Can you explain why canned fruit frequently "sours" while preserves do not?

*b. Effect of temperature upon growth.*—Fill each of three test-tubes one-third full of Pasteur's solution with sugar, into each put a drop of fluid yeast, and close the mouth of the tube with a plug of cotton-wool. Set the first tube in some place, as in the water-bath, where the temperature can be kept constantly at about 35° C. Boil the contents of the second tube for two or three minutes, then set this tube with the first. Put the third tube on a block of ice or in a dish of ice-water. Examine each tube two or three times a day for several days and note what takes place. Explain your results. What is the effect of a very high temperature, *i. e.*, boiling? Of a very low temperature, *i. e.*, freezing?

*c. Effect of light upon growth.*—Prepare two test-tubes with Pasteur's solution and a drop of yeast as in *b*. Wrap one tube with thick paper so as to exclude the light. Leave the other unwrapped. Set the two tubes side by side in a window where

Copy { they will be exposed to the sunlight. Examine the tubes from time to time for two or three days. In which tube does the yeast grow better? Has sunlight any effect upon the growth of yeast?

d. *Nature of the gas given off by the growing yeast.*

1. Take two test-tubes, a perforated cork which will fit one of the test-tubes, and a U-shaped glass tube, one branch of which is passed through the perforation in the cork. Fill the first test-tube one-third full of clear baryta water, or lime-water may be used if necessary; into the second test-tube put some baking-soda or bicarbonate of soda (which is a combination of sodium and carbon dioxide, or "carbonic-acid gas"); pour into ~~the second test-tube~~ a few drops of dilute muriatic or of dilute sulphuric acid; insert the cork at once in such a manner that the end of one arm of the U-shaped tube is *above* the surface of the fluid in the test-tube; hold the first test-tube in such a manner that the end of the other arm of the U-shaped tube will extend *below* the surface of the baryta water in this test-tube. The gas (*carbon dioxide*) formed in the second test-tube by the action of the acid upon the soda will thus be carried by the U-shaped tube over into the first test-tube, where it will bubble up through the baryta water. What change takes place in the baryta water?
2. Pour some clear baryta water into a watch-glass, and, holding the mouth close to the surface of the liquid, breathe heavily through the mouth upon the surface of the water. What change

takes place in the water? Is this result at all like that obtained in the first experiment? Explain.

3. Take two test-tubes, prepare the first with baryta water as in the first experiment; fill the second test-tube about half full of yeast which is actively giving off bubbles of gas, connect the two tubes as before so that the gas will bubble up through the baryta water. If the gas stops forming, add a little sugar to the yeast. Does the baryta water change as in the first experiment? As in the second? Is, then, the gas which is given off by a liquid in which yeast is growing the same as the gas formed by the action of an acid upon bicarbonate of soda, and also the same as that exhaled from the human lungs?
4. Put a half-teacupful of actively growing yeast into a loosely corked bottle, and set the bottle in a warm place for an hour or so. Is the cork blown out? Why? Prepare another bottle in a similar manner, but tie the cork down with a cord or wire. Do bubbles of gas still form in the fluid? What do you conclude, then, regarding the *energy* with which the gas is formed in the fermenting liquid, in spite of the pressure on its surface due to the accumulation of gas in the upper part of the bottle? The pressure of the accumulated gas may be measured directly by means of a manometer. This consists of a U-shaped tube partially filled with mercury. First mark the level of the mercury in the two branches; then, by means of a rubber tube, connect one branch with a glass tube run through the stopper of the bottle of yeast.

The gas will come through the glass tube and the rubber tube over to the surface of the mercury in one branch of the manometer, and push down the mercury on that side and raise that on the other. Measure the height of the mercury, and calculate the pressure needed to raise the column of mercury to this height.

*e. Formation of alcohol.*—Grow some yeast in Pasteur's solution with sugar, in a flask closed with a cork through to the lower surface of which passes a glass tube about eight inches long, bent downward at an angle of about  $30^{\circ}$ . When the formation of gas has entirely ceased in the flask, set the latter on a water-bath and, at the lowest temperature possible, distill the fluid into a test-tube held at the end of the bent tube. When the test-tube is about half full, remove the stopper and bent tube from the flask, insert them into the test-tube, and redistill the contained fluid, collecting the first few drops in a watch-glass. Do they give off the odor of alcohol? Apply a lighted match to the fluid in the watch-glass to see if it ignites and gives the pale blue flame characteristic of burning alcohol.

*f. Chemical reaction of fluid yeast.*—Dip the end of a strip of blue litmus paper (which turns red when placed in an acid) into some fluid yeast. What change takes place in the color of the paper? Is fluid yeast acid or alkaline in its nature? Note, also, the odor of the fluid.

*g. Temperature of yeast during growth.*—Take two

Florence flasks (bottles may be used), and into each put about one litre of Pasteur's solution with sugar. Into the first put also 200 c.c. of fresh yeast. Close the mouth of each flask with a plug of cotton-wool. Wrap each flask in a cloth, or, better, in cotton, and put them side by side in a box to protect them from draughts. When fermentation becomes vigorous in the first flask, as will be indicated by the formation of bubbles of gas, take the temperature of the liquid in each flask with a good thermometer and compare the results. In which flask is the temperature higher? Let the flasks stand until about ten or twelve hours after the formation of bubbles has ceased in the first flask, and then take the temperature again. Was the difference in temperature first noticed due to the *presence* of yeast or to its *growth*? Why?

- h. Effect of filtered yeast upon a fermentable fluid.*—Sterilize a small porous porcelain cup, such as is used in batteries, by boiling it in water for several minutes. Set the porous cup into a teacup which has also been boiled, and fill the porous cup about half full of fluid yeast. In a short time some of the fluid part of the yeast will filter through the porous cup, leaving the solid part (yeast cells) behind, and will be caught in the teacup. Fill a test-tube one-third full of Pasteur's solution with sugar, plug the mouth of the tube lightly with cotton-wool, and sterilize the contents by boiling for about five minutes over the flame of an alcohol lamp or a Bunsen burner. Allow the fluid to cool, then with a glass rod,

which has been sterilized by being passed through the flame of the lamp, take up a few drops of the fluid in the teacup, and mix them with the Pasteur's fluid in the test-tube. Set the test-tube in a warm place, *e. g.*, 35° C., for a few hours, examining from time to time to see if bubbles of gas are formed. Is the actual presence of yeast cells necessary to produce fermentation, or is there something in the fluid in which yeast cells have lived which will bring about that change? If the latter, whence does that something—*i. e.*, *enzyme* or *ferment*—probably come?

*i. Reproduction.*

1. *Budding* or *gemmation*. — Examine cells from each of the cultures made in the experiment on the effect of food-supply upon growth. In which have the cells the largest number of buds? In which the smallest? How many buds may a cell have? Do all the cells have buds? Can you always distinguish which is the "parent" and which is the "bud"? Are the buds always formed on the same part of the cell? What are the steps in the process of the formation of a bud? What is the difference between reproduction by budding and reproduction by fission? Is the difference fundamental?

Make drawings to show how buds are formed and how they are attached.

2. *Endogenous spore-formation*. — Make a slab of plaster of Paris about one-fourth of an inch in thickness and about two inches square. Upon one face of this paint with a clean camel's-hair

brush a thin layer of yeast taken from a supply which is actively growing. Put the slab under a bell-jar or a tumbler with some pieces of wet blotting-paper to keep the air moist. With the point of a penknife or scalpel remove some of the cells each day for eight to fourteen days, and examine under the high power of the microscope for spores formed inside the cell. It is well to make several such cultures, as some are almost certain to be spoiled by the growth of moulds and bacteria. Compare with the process of budding. Do you find any relation existing between nutrition and reproduction? Do you find any evidence of the conjugation of individual plants?

Make drawings illustrating endogenous spore-formations.

*General Questions.*—Compare the results obtained in all of your work on this plant, and tell what yeast forms from its food.

Of what use is yeast in bread-making? In brewing? Is yeast a “cultivated plant”? If so, how is it cultivated and what is the “soil” upon which it grows?

**EXAMPLE 2.—Green Slime (*Protococcus Sp.*)**

*Material.*—Specimens of green slime, or of other unicellular green plants which are so closely related to the form selected as to be equally serviceable, may be found almost everywhere, forming a greenish, slimy ooze on the surface of the mud and stones in the bottoms of slow-running ditches, on the trunks of trees near the ground, on the stones or bricks in the foundations of



buildings, on the outside of damp flower-pots, etc. They will be most easily found after a few days of rain; at other times their bright green color is likely to be dingy and not so noticeable. If the plants be found on a tree, cut off pieces of the bark and put them in a saucer with a little water to moisten the bark, cover with a tumbler, and set them in a window where they will have the sunlight. In this manner a supply of fresh specimens may be kept for a long time and the various stages of growth studied from time to time. This method of procedure is advisable, as it is sometimes impossible to find material showing both the vegetative and the reproductive stages of the plant. Besides the specimens, the student will need strong iodine, alcohol, seventy-five per cent. sulphuric acid, dilute chlor-iodide of zinc, and a compound microscope; also some cotton fibres, which are best obtained from a cotton-boll, as they have then passed through none of the processes incident to manufacture, and are in a perfectly natural condition. If the boll cannot be obtained, fibres from a thread or from a piece of cotton cloth may be used.

*Method of Examination.*—Before resorting to the microscope examine, as directed on page 41, the naked-eye characters. If the material be obtained from a very damp situation, a small piece of the mud or of the film of slime may be put upon the slide, in a drop of water, and torn to pieces with the dissecting needles, so as to separate the cells of the plant from the tangle of thread-shaped algæ and fungi, associated with which the green slime almost always grows. If the material be dry and powdery, as it is likely to be if obtained from bark in dry weather, a little of the film may be scraped into a drop of water on a slide by means of

the needle point or a scalpel. The cover-glass may then be put on and the specimen examined as usual.

#### MORPHOLOGY

##### *Naked-eye Characters :*

- a. *Occurrence.*—In what other places than those mentioned above do you find the plant? If found in ditches, do you get it most abundantly in muddy, sandy, or rocky places? If on flower-pots, on the lighted or shaded side? If on trees, does it grow better on those with rough or on those with smooth bark? Why? Does it show preference for the north, east, south, or west side? Why? Is the film even in thickness, or do you find marked variations in this respect?
- b. *Color.*—What is the usual color? Are specimens from different situations always of the same color? If you find variations, how do you explain them? Put under a tumbler or a bell-jar and keep moistened a piece of dry bark bearing green slime. Does the color change at all? Put into a small bottle of alcohol a piece of clean bark upon which green slime is growing and leave for a few hours, then remove the bark from the bottle. What change, if any, has taken place in the color of the green slime? In the alcohol? Explain.
- c. *Structure.*—Scrape the film off a piece of dry bark. Does it adhere firmly to the bark? Does it separate into large or small flakes? Do the flakes hold together well, or do they break up into small particles? Scrape a film off a piece of

damp bark, examine, and account for any differences you may find.

*Microscopic Characters:*

Examine first with a low, then with a high power specimens prepared as directed.

*A. Vegetative Condition.*

- a. *Arrangement.*—Do you find groups of cells? Single cells? What various numbers of individual cells do you find constituting different groups? Are the cells arranged at all as in yeast?

Draw single cells and groups.

- b. *Shape.*—What is the shape of a single cell? Is the shape symmetrical or irregular? Do you find any noticeable variations? How is the shape of the individual cell modified where the cells occur in groups? Compare the shape with that of the yeast cell. Does it have a greater variety of shapes than the latter? How do you account for them? Does the cell have outgrowths or projections of any kind, such as roots? How does green slime remain attached to the surface of the bark or stone? Has it any organs of motion? Does it resemble in shape any of the *Protozoa*?

Draw several specimens showing marked variations from the normal shape.

- c. *Size.*—Do the cells vary much in size? Measure several and find the average dimensions. How do they compare in size with yeast? With the slipper animal? With the red corpuscles of the frog?

- d. *Color.*—What is the color as seen under the micro-

scope? Is it different from that seen with the unaided eye? Is the color evenly distributed throughout the cell? Do you find cells showing more than one color? Examine some cells taken from the piece of bark which was put in alcohol, and compare them with the fresh cells.

*e. Structure.*

1. **The cell-wall.**—Is its surface smooth or rough? Is the wall thicker or thinner than that of a yeast cell? Are there any variations in thickness? Is the wall colored?
2. **The cell-contents.**—Can you distinguish protoplasm in the cell? Nucleus? Vacuole? Chromatophores (**chlorophyll bodies**, or green masses of protoplasm)? Run a drop of strong iodine under the cover-glass. What effect has it on the protoplasm of the cell? Does it show the presence of starch grains in the cell? What effect has it on the cell-wall? Apply to the same specimen a drop of seventy-five per cent. sulphuric acid. Do you notice any change in color in the cell-contents? In the wall? Make a fresh preparation of the plant and apply a drop of dilute chlor-iodide of zinc. How are the cell-contents and the wall affected? Let the specimen stand for an hour or two, then examine again. Do you notice any important changes, especially in the chromatophores?

Make drawings to show the structure of a single cell.

*f. Tests for cellulose.*

1. Put some cotton fibres (which are almost pure cellulose), into a drop of water on the slide, put

- on the cover-glass, and examine. Note the shape, color, and structure of the fibres.
2. Run a drop of strong iodine under the cover-glass. What is the effect upon the fibres? Compare with the same treatment of the cell-wall of green slime.
  3. Follow the iodine with a drop of seventy-five per cent. sulphuric acid. What change? Compare with the preceding experiment and with the color of the green slime after such treatment. The preceding and this experiment together constitute the *iodine-sulphuric acid test for cellulose*. How does it differ from the test for starch?
  4. Mount some cotton fibres as in the first experiment, then run under the cover-glass a drop of dilute chlor-iodide of zinc. What is the effect upon the fibres? Compare with that produced by iodine and sulphuric acid used together, and with the effect upon the wall of the cells of green slime. Is the cell-wall of green slime composed of cellulose?

*B. Reproductive Condition.*

In addition to the motionless or *vegetative* cells, certain others, the **zoöspores**, will probably be found actively moving about. If they are not found in the material when first gathered, they are likely to appear if water containing the vegetative cells be left exposed to the sunlight for a few days. The zoöspores are of two sizes, the larger being called **macrozoöspores** and the smaller **microzoöspores**. Examine the shape, size, and structure of each. Try to find vegetative cells whose contents are dividing into 2-4

parts or macrozoöspores, or into several parts or microzoöspores. Note the cilia with which the zoöspores of each kind are provided. How many cilia do you find on each? Are they of equal length? If the cilia are indistinct, the cells may be stained with dilute iodine. Can you discover a cell-wall around the protoplasm of the zoöspores? Do you find that the zoöspores unite? Is the movement of the cells rapid or slow? Why should these reproductive cells be motile, while the vegetative cells are motionless? Look for zoöspores which have come to rest. Can you determine whether or not they have a cell-wall, using reagents if necessary? Can you distinguish such a zoöspore from a vegetative cell? In what ways do the zoöspores resemble any of the *Protozoa*, especially in structure? Do they exhibit any nervous properties, such as automaticity, irritability, etc.? Examine the dish of water in which the zoöspores were found, or transfer water containing them to a dish and place the dish in the sunlight for a few hours, then examine a drop of water taken from the side of the dish *towards* the light and another from the side *away* from the light. Which drop contains the greater number of zoöspores? Having obtained a definite answer to this question, stir the water thoroughly, so as to distribute the cells evenly through it, then transfer the dish to a place out of the direct sunlight, preferably several feet from the window, and let it stand for several hours. Then examine two or more drops taken from opposite sides of the dish as before. Do you find any difference in the position of the zoöspores? If so, how do you explain it?

Make drawings to illustrate as many as possible of the facts you have learned concerning the reproductive stage of green slime.

*General Questions.*—In what ways may green slime be dispersed? Is green slime dependent upon “food solutions” for its nourishment? If not, how can it, having no roots, get food? Which do you regard as the higher plant, green slime or yeast? Why?

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### B.—MULTICELLULAR PLANTS

The following examples are cells from these plants, isolated from the body:

**EXAMPLE 3.—Spores of Fungi** (*Penicillium*, *Eurotium*, *Agaricus*, etc.)

*Material.*—If no bread, cake, jam, or leather bearing mould can be obtained, a slice of bread or cake or a few dried prunes may be put on a saucer under a bell-jar or tumbler with a few scraps of moist blotting-paper, and set away in a warm place. Moulds will usually appear in the course of a few days. Specimens may also be obtained frequently from the top of canned fruit and preserves. In case mouldy substances fail, the top of a ripe mushroom or toadstool which may be had at green-houses at almost any season will do as well. Iodine, acetic acid carmine, magenta, Pasteur’s solution with sugar, compound microscope, bell-jar, and the moist chamber will be used in the examination of the material.

*Method of Examination.*—Hold a piece of the mouldy

material over a drop of water on a slide, and by gently tapping the mouldy substance dust some of the spores into the drop. Put on the cover-glass, supporting it at one edge by a piece of hair, and run a drop of fifty per cent. alcohol under the cover-glass, as water alone does not wet the surface of the spores.

#### MORPHOLOGY

*a. General Structure.*—To the unaided eye what appearance have the spores? Are they numerous or few? What color has a mass of spores? Examine first with a low, then with a high power. What is the shape of the spores? Are they separate or connected? Can you distinguish, with or without the use of iodine, acetic acid carmine, magenta, etc., whether or not the cells have wall, protoplasm, chromatophores, nucleus, etc.? In what respects do these spores resemble yeast cells? In what respects do they differ from the cells of green slime? How do you account for the wide dispersal of the spores of moulds?

Make drawings to illustrate shape, size, and structure.

#### PHYSIOLOGY

*a. Germination.*—Dust some spores into a drop of water and with the point of a needle transfer a small portion, so as to get as few spores as possible, to a drop of Pasteur's solution with sugar in a moist chamber, and examine at intervals for several days. Unless great care is taken foreign organisms—bacteria, etc.—are likely to interfere with the growth of the spores. The following method of preparation will probably give good results:



Boil a small amount of Pasteur's solution or of dilute prune-juice, and with a glass rod, which just before using has been passed slowly through the flame of an alcohol lamp, transfer a drop of the fluid to the surface of a cover-glass which has likewise been passed through the flame; have ready the glass slide, which previously has been sterilized in the same manner, and lay on it the card-board cell or glass ring, which has been sterilized in boiling water; invert over the cell as quickly as possible the cover-glass with the hanging drop, and after making sure by microscopic examination that spores, drop, etc., are in good condition, set the slide away under a bell-jar in a warm place. Note how the spores sprout. How long before the process begins? What is the first indication that a sprout is forming? How does the size of the tubes put forth compare with that of the spores? How do you account for the difference?

Make drawings to illustrate different stages of germination.

*General Questions.*—Upon what do the spores depend for their dispersal? How do you account for the moulding of bread, pastry, leather, ink, etc., in warm, damp weather? Why does it not also happen as frequently in cold weather? How is it possible for fruit in closed cans to become mouldy?

#### EXAMPLE 4.—Pollen Grains

*Material.*—The best plan will be to get pollen from a number of flowers and make a comparative study. It

is immaterial whether the flowers be wild or cultivated, but as the latter are always to be had a few common kinds may be mentioned as suitable: Sweet-pea (*Lathyrus*), Evening Primrose (*Oenothera biennis*), Willow-herb (*Epilobium*), Fuchsia, Hollyhock (*Althaea rosea*), Mallow (*Malva crispa*), Onion (*Allium*), Tulip, Tiger-lily, Japanese Lily, Hyacinth, *Gloxinia*, Poppy (*Papaver*), Pansy (*Viola tricolor*), or Chicory (*Cichorium intybus*). The anthers of the flowers should be examined, with a hand-lens if necessary, to see if they have opened. If so, the pollen is in proper condition for study, and will usually appear as a yellow, white, or brown powder.

In the examination may be used iodine, Schulze's solution, potash, acetic acid carmine, and sugar.

*Method of Examination.*—With the point of a needle remove a little of the pollen to a slide, and examine first in the dry state—*i. e.*, without the drop of water and the cover-glass. Afterwards prepare the pollen in the drop of water in the usual way. Note particularly the difference in the appearance of the same kind of pollen examined in each of these ways.

#### MORPHOLOGY

*a. General Structure.*—Do you find that the individual pollen grains are visible to the unaided eye? Do you find any variety of shape, size, color, and structure? Do you find that the color of the individual grain is the same as that of a mass of the same grains? What do you find in the way of markings, grooves, wing-like expansions, points, etc., on the wall of the grains? Can you

determine the use of any of these? What means of protection from being dried, eaten by insects, attacked by parasitic fungi, etc., do the pollen grains exhibit? Examine the stigmas or tips of the pistils of the flowers of several different kinds of plants, and note the presence of pollen grains in some if not in all cases. Do you find in any case that the pollen grain has means of its own for getting from the anther to the stigma—*i. e.*, has it any organs of motion? If not, how does it make its way from one part of the flower to the other? Examine the grains for the presence of protoplasm, nucleus, chromatophores, oil drops, etc., using reagents if needed.

Draw several specimens to show the variations in shape, size, and markings.

#### PHYSIOLOGY

- a. *Germination*.—Make a dilute syrup or nectar by placing seven or eight crystals of “granulated” sugar in a drop of water on the slide. When they have dissolved, dust into the drop a few grains of pollen from some of the flowers named above, preferably the Sweet-pea. Let the grains remain in the syrup for several hours, examining from time to time to study the formation of the pollen tube. Note its size and rate of growth in different grains. Compare with the germination of the spores studied in Example 3, and note all of the resemblances and differences that you can discover. Much more successful cultivations may be made in a hanging drop in a moist chamber. Pollen grains which have germinated under natu-

ral conditions may be found by examining the stigmas of various flowers with a hand-lens, especially lilies, hyacinths, fuchsias, etc., and having found a stigma to which grains are attached, removing them to the drop of syrup prepared as above.

Do you find variations in the time it takes seemingly mature grains to germinate? Is the pollen tube single or many celled?

Make drawings to illustrate the different stages of growth in pollen grains of the same kind and of different kinds.

**EXAMPLE 5.—Water Silk (*Spirogyra* Sp.)**

*Material.*—The study of this plant is introduced here because its cells are so large and lend themselves so readily to manipulation that they form most desirable material for the study of the structure and functions of the plant cell.

These plants may be obtained in abundance during the warm season of the year in almost any stagnant pond or along the edges of slow-flowing streams. They form the yellowish-green, frothy scum popularly called "frog-spittle" or "pond-scum." Various species are usually found growing together, along with other fresh-water algæ. Almost any species will serve for study, but the larger kinds are especially favorable. They may be kept for examination in winter in aquaria with opaque sides, which should be placed in sunny windows, be supplied with fresh water from time to time, and kept partially covered to exclude the dust. In changing the water be careful to disturb the plant as little as possible. If it be found that the water is becoming infested with too many micro-organisms, transfer some

of the material to a jar of fresh water. Under such conditions of cultivation the plants will go through their fruiting stages nearly all winter. If it be impracticable to keep living plants for study, specimens may be preserved in fair condition, though the color will be lost, in a mixture of equal parts of water, glycerine, and alcohol.

The following study requires the use of the hand-lens, compound microscope, fine forceps, seventy-five per cent. sulphuric acid, Schulze's solution, dilute iodine, picric acid, strong potash solution, ninety per cent. alcohol, carmine, magenta, two per cent. salt or sugar solution, ten per cent. salt or sugar solution, twenty per cent. salt or sugar solution, a vial or test-tube, distilled water, Sachs's food-solution, ice, and a saucer.

*Method of Examination.*—The plants should first be studied in their native pool, their surroundings and habits noted, etc. Then examine some as they grow in an aquarium. Then, with the forceps, transfer one or two filaments to a porcelain bowl or saucer in which the dark green of the plants will show well against the white background formed by the saucer. For the microscopic study only two or three filaments should be placed on the slide, where they may be studied with the hand-lens and with both low and high powers.

#### MORPHOLOGY

##### *A.—Vegetative Condition.*

##### *I. Naked-eye characters.*

Examine some of the plants as they grow in the aquarium, particularly the position occupied by the mass after exposure to sunshine for a few

hours, and again the next morning before the sunlight reaches the aquarium, and by individual filaments. Put several filaments into a saucer of water and note their shape, color, length, and diameter. What variations do you find in these features? If necessary use a magnifying-glass. Does the plant grow attached to a substratum? Can you see any branches? Roots? Why is the plant an inhabitant of *stagnant* water? Feel of the plants. What significance in the common names?

## II. *Microscopical characters.*

Mount two or three filaments in water and examine first with a low, then with a high power, and note:

a. *The shape of the filament.*—What is it? Is its diameter uniform? What is the shape of the ends? Can you detect any branches or roots? Has it a “root end” and a “stem end”?

b. *The structure of the individual filament.*—Note that the filament is composed of cells. How are the cells arranged? What is the shape of a single cell? What relation between its length and breadth? Are all of the cells of this shape? How are the cells connected? Is there any difference between the two ends of the filament? Be careful not to be deceived by injured cells, which are frequently to be found at the end of the filament. Look for a transparent, slimy coating covering the filament. This is seen best just before a drop of stain reaches the filament. How

do you account for the slippery feel of the plant?

c. *The structure of the individual cell.*

1. The **cell-wall**.—What is the shape of the side-wall? Of the end-wall? What is the color of the cell-wall? Which is the thicker, the side-wall or the end-wall? Do you find any openings in either wall? Markings of any kind on the wall? Run a drop of strong iodine under the cover-glass, then a drop of seventy-five per cent. solution of sulphuric acid, and note the effect upon the cell-wall. Of what is it composed? Try also Schulze's solution. Treat a fresh preparation with strong potash solution, and note the *stratification* of the wall. How do you account for this appearance?

Take another fresh preparation and examine

2. The **chromatophores** or **chlorophyll bodies**.  
—What is their arrangement? General shape? What is the shape of their margins? How many in a cell? How many turns does each make? In what part of the cell do they lie? Do they extend from one cell into the next? What is their color? Are there any other colored bodies in the cell? Do these chromatophores in any way resemble the green part of the cell of *Protococcus*? To what is due the color of the plant as a whole? What significance in the scientific name of the plant? Put some filaments into strong alcohol and examine them after a few hours. What change in the chromatophores? In the alcohol? Explain. Stain some of the filaments with carmine or

magenta, which are substances which stain protoplasm especially. What is the effect on the chromatophores? On the cell-wall? Of what substance are the chromatophores composed?

3. The **pyrenoids** and **starch grains** (seen in the chromatophores).—Apply picric acid to make the pyrenoids more plainly visible. How are the pyrenoids arranged? What is their color? Shape? Run a drop of iodine under the cover-glass and note the position and number of the starch grains. What is their relation to the pyrenoid? Do they at all affect the shape of the chromatophore? Examine some of the cells treated with alcohol in the second experiment. Does carmine or magenta stain the pyrenoid? Of what substance is the pyrenoid composed? Treat with iodine some other cells which have been in alcohol. Is starch still present in these cells? Explain. Do you find starch grains anywhere else than in the neighborhood of the pyrenoid?
4. The **nucleus**.—What is its position in the cell? Shape? Color? Can you find the nucleolus? If so, what is its position? Shape? In which cells is the nucleus seen most distinctly? Notice the strands of protoplasm radiating from the nucleus. To what do they run? How many can you find? Examine the nucleus in cells treated with alcohol, iodine, magenta, etc.
5. The **primordial utricle** or **protoplasmic sac**.—Try to see this (without using reagents) as a thin film of protoplasm lining the cell-wall. If unsuccessful, *plasmolyze* the cell by running a



drop of ten per cent. salt or sugar solution under the cover-glass, and note how the protoplasm shrinks away from the cell-wall. What is the cause of the shrinkage of the protoplasm when the cell is plasmolyzed? What properties of protoplasm does the experiment illustrate? What becomes of the chromatophores? What change takes place in the cell-wall? Try plasmolyzing the cell with a twenty per cent. solution of salt or sugar. What difference in the action of this and of the weaker solution? Plasmolyze with a two per cent. salt solution. As soon as the protoplasmic sac shows plainly, remove the salt solution and replace it with water. Does the sac expand until it again fills the entire cell? Explain. Explain all of the changes noticed.

6. The **vacuole**.—Note that a large part of the cell is occupied by the vacuole. What change takes place in this when the cell is plasmolyzed? Explain. Examine several cells to note the variation in the size of the vacuole. Explain.

Draw a single filament seen from the side to show its structure, ends, etc.; a single cell seen sidewise to show its contents, structure of wall, etc.; the same cell seen from the end with contents in natural position; another cell plasmolyzed.

*B.—Reproductive Condition.*

- a. *General appearance*.—Can you, without using a microscope, distinguish this condition in any way, as by difference in color of the mass, length of filament, position in aquarium, etc., from the vegetative condition?

- b. The structure of the fruiting filament.*—Are there any noticeable changes in shape or size as compared with the vegetative filament? In color? Do the cells have the usual contents? Can you find the chromatophores, nucleus, etc., in each cell of the fruiting filament? Is starch present?
- c. The position of the fruiting filaments.*—In what position do the fruiting filaments lie? What determines this position mathematically? Physiologically? Do the filaments touch at all points? Do you find a filament in any part of its course in contact with more than one other filament?
- d. The conjugating tubes.*—How many does each cell have? Do all of the cells have them? How do they compare in shape and size with the rest of the cell? Look for tubes in various stages of development. How are they formed? Are they a mere “bulging out” of the cell-wall, or are they true outgrowths of the same? Do they always grow from the same side of the cell? What is their structure? Do they show the same structure and composition as the cell-wall? What determines their place of formation physiologically? Are the filaments actually in contact before the tubes form? Are they open at the outer end from the first stages of formation? If not, when do they become open? Do the tubes in conjugating filaments appear to be permanently grown together, or are they only in temporary contact? What becomes of the tubes after the zygosporangium is formed?
- e. The zygospores.*—Do they occur in both filaments?

Are they formed in each cell of the fruiting filament? What determines their place of formation? Is there any visible difference, such as would indicate sex, between the two filaments whose cells conjugate to form the zygospores? Look for zygospores in various stages of development. Can you find the different stages of this process exhibited in a single filament? What are the details of the process? What is the shape of a ripe spore? Structure? What is left in the conjugating cells after the zygospore is formed? What significance in the name "zygospore"? Can the spore pass out of the cell through the conjugating tube? If not, how can the spore get out? After the formation of the spore is the cell living or dead? Notice that the entire cell-contents which at one time is "vegetative" becomes "reproductive."

Draw a number of cells to illustrate the facts learned.

#### PHYSIOLOGY

In addition to the physiological topics studied in the examination of the reproductive stage of the plant, the following may be investigated:

##### *A.—Formation of Starch (Assimilation).*

###### *I. Effect of light.*

- a.* Take some filaments of *Spirogyra* whose cells are known to contain starch, place the filaments in a dish of water, and set the dish in a warm, dark place for two or three days, or until microscopic examination shows no starch to be present, being careful to prevent the total evaporation of the

water in the dish. At the end of the time stated examine some of the filaments under the microscope, and note the changes in the chlorophyll bodies and starch grains. What has become of the starch?

- b. Expose some of the filaments used in the preceding experiment to the bright sunlight for fifteen minutes to two or three hours according to the intensity of the light, then test with iodine. Is starch present? Explain. Note the bubbles of gas given off by plants exposed to the sunlight. Compare these with the plants growing in ponds. What causes the plants to float at the surface of the water when exposed to the sunlight? What advantage in this? Why do they sink down in the water at night? What advantage in this? Is it possible that the slimy excretion on the filaments may assist in any way to float the plant? Why?

What conclusions do you draw from these experiments?

## *II. Effect of carbon dioxide.*

- a. Boil some distilled water to drive off any carbon dioxide it may have absorbed, cool the water, place some of the filaments used in *I. a.* in a vial, fill the vial completely with the cooled water, and set it in the sunlight for fifteen minutes to an hour, then test for starch as in *I. b.* What results?
- b. Take some more of the filaments used in *I. a.*, and put them in a vial of hydrant water, which usually contains carbon dioxide, and set the vial alongside of *II. a.* for the same time. Compare

results. Does starch form more or less rapidly in this case than in *II. a.* ?

What conclusions do you draw from these two experiments ?

*B.—Growth.*

- a. Put some filaments of *Spirogyra* into a bottle containing distilled water, and set the bottle in the sunlight.
- b. Put some filaments of *Spirogyra* into a bottle containing Sachs's food-solution for green plants. Let the two bottles stand in the sunlight for a week or two, then compare the results found in each experiment. In which bottle do the plants grow better ? Explain.

*C.—Cell-formation.*

- a. Place some actively growing filaments of *Spirogyra* in a cold place (almost freezing) overnight. Examine them early next day for cells in the process of division. What are the first steps in the process ? What changes take place in the nucleus ? In the chromatophores ? In the cell-wall ? Is the process confined to any particular part of the filament ? To particular cells ? How long does it last ? Can you now account for the difference in thickness between the end and side walls ? Would you consider cell-formation to be a kind of reproduction ? Why ? In what respects is cell-formation in the plant different from spore-formation ?

Make drawings showing the various stages of cell-formation seen.

*General Questions.*—Is a filament of *Spirogyra* a sin-

gle plant consisting of many similar cells, or a colony of unicellular plants? Why? What do you regard as the main points of difference between *Spirogyra* and *Protococcus* considered both morphologically and physiologically? Between yeast and *Spirogyra*?

Review all of your studies of animal and plant cells. Do you consider the *cell* to be an *organized body*? If so, why?



**PART II**

**THE BIOLOGY OF THE ANIMAL**





## THE BIOLOGY OF THE ANIMAL

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### SPONGES

#### EXAMPLE 1.—**Skeleton of Tillet Sponge** (*Euspongia Sp.*)

*Material.*—Specimens may be obtained at any drug store. It must be borne in mind that these are almost invariably imperfect, since in preparing the sponge for market it is trimmed more or less to give it a symmetrical shape. The trimming is usually confined to the base, which contains a collection of débris consisting of sand, small pebbles, pieces of shells, etc., whose presence would affect the market value of the sponge. Branched forms also are clipped to make them into a more convenient shape. Students will readily detect the places which have been trimmed by the fact that the surfaces are smooth and even. The small sponges used for washing slates are generally pieces sheared off larger sponges. It is well to have for comparison some fine surgeon's sponges as well as the coarser kinds used for washing carriages.

Besides the specimens the student will need a magnifying-glass, compound microscope, scissors or sharp knife, and a tumbler of water.

*Method of Examination.*—The sponge skeleton should

first be examined entire, then with a sharp knife or scissors be cut into several sections parallel with the base; a second specimen should be cut into vertical sections. Small scraps may be torn off the tips of the canals and examined under the low power of the microscope.

#### MORPHOLOGY

- a. Shape.*—Do you find that all of several specimens have the same general shape? What is it? Do you find any noticeable variations from this? If so, can you suggest any reasons for such variations? Can you readily detect an upper and a lower side of the sponge body? How? Right and left? How? Notice the elevations of the general surface. Have they any relation to the large canals which run through the mass of the sponge skeleton?
- b. Size.*—What is the average size of a number of specimens? Do you consider this the average size attained by this kind of sponge, or is it merely the *marketable* size? Why?
- c. Color.*—What is the usual color of a toilet sponge? It must be remembered that many sponges are bleached during the process of preparing them for market. Such sponges usually have a bright-yellowish tinge.
- d. Elasticity and porosity.*—Squeeze a dry sponge tightly in the hand. Does the sponge regain its original size? Does it show any tendency to do so? Now put the same sponge into a dish of water

and note the change. How do you account for it? Is a wet sponge more elastic than a dry one? Soak a sponge thoroughly in water, then squeeze out as much as possible of the water into a dish and measure the amount.

*e. Structure.*

1. The **oscula** (large openings on the upper surface of the sponge).—How many on your specimen? What is their shape? Size? Note that each osculum is the outlet of a canal which runs down into the body of the sponge. Look down into an osculum and notice the numerous smaller canals which open into the large one. Notice also that the skeleton becomes thinner and less compact at the margin of the osculum.
2. The **canals**.—With a sharp knife or a pair of scissors cut through the skeleton so as to divide at least one of the large canals lengthwise. How far into the body does the canal penetrate? Does it decrease or increase in size as it approaches the osculum? Do any of the large canals unite directly or are they connected by smaller canals? Do the walls of the canals have the same appearance as regards smoothness, texture, color, etc., as the surface of the skeleton?

Make outline drawings showing the sponge as seen from the side and from the top, indicating the position of all of the large oscula; also a drawing of a section showing the course of some of the principal canals.

3. The **fibres**.—Look closely at the surface of the

sponge skeleton and notice that it is constructed of fibres united together. Can you detect with or without a magnifying-glass whether these fibres differ in size, color, arrangement, etc., in different parts of the skeleton? If you find any differences, how do you account for them? Tear off a small scrap of the skeleton and examine under the low power of the compound microscope. What arrangement of the fibres do you find? Does this give you any clue to the reason why a toilet sponge can absorb so much water? Look again for differences in the shape and size of fibres. How are the fibres held together? Do you find any traces of joints? Do you find any foreign bodies, as grains of sand or pieces of shell firmly attached to the fibres or incorporated in them? If so, how do you account for their presence?

Draw a magnified piece of sponge to show the shape and arrangement of the fibres.

**EXAMPLE 2.—Fresh-water Sponge (*Spongilla* Sp.)**

*Material.*—This sponge is common in many lakes and rivers throughout the country. It forms dark-green patches on the surfaces of submerged rocks, logs, pieces of bark, etc. It frequently grows in large, branched clusters from a few inches to a foot or more in length, the branches sometimes being as large around as the thumb. Owing to the color and shape of these branched forms they are often mistaken for water-weeds or masses of algæ. The difference can be told at once even by an inexperienced

person, for, if a piece of sponge be gently pressed between the thumb and finger, it crumbles to fine particles which feel gritty. Further, a close examination, with or without a hand-lens, will show numerous fine openings scattered over the surface if the specimen really be a sponge. In collecting *Spongilla* put the specimen together with the object to which it is attached into a pail with an abundance of water, and handle as little and as gently as possible. By changing the water three or four times a day specimens may be kept alive for several days. A good plan is to set the pail where a slow stream of water may flow through it. The following are especially good places to find specimens: the rocks at the foot of a mill-dam, the sluice-ways and gates of a mill, and the under side of rocks and logs in swift-flowing streams. There is almost always a possibility of finding them in clear, rapid streams, but never in water which is permanently muddy. The best specimens are to be found between July and December.

To prepare alcoholic specimens for preservation drop pieces of the branches of the living *Spongilla* into about fifteen or more times their bulk of absolute alcohol and change the alcohol at the end of two or three hours. The color of the specimen is altered, but the structure is well retained.

In the examination will be used the hand-lens, compound microscope, acetic acid carmine, hydrochloric acid, fine bristles, chalk or indigo, and forceps.

*Method of Examination.*—Living *Spongilla* should be examined where found if practicable. If removed to the laboratory the examination should be made at the earliest possible moment, as it is very difficult to keep

specimens alive. Alcoholic material may, without serious injury, be transferred to a dish of fifty per cent. alcohol, but should not remain more than an hour or two, otherwise maceration will take place.

#### MORPHOLOGY

- a. Shape.*—What is the shape of the branched form? Does it have a single trunk? How many branches has it? Does each branch remain separate—*i. e.*, not connected to others—throughout its course? If not, how are the branches connected? What is the shape of the specimen at the point where it is attached to its support? Does it have any root-like outgrowths? What shape as regards outline and thickness do the flat forms of *Spongilla* take?

Sketch several specimens in outline.

- b. Size.*—How high is your specimen? What is its extreme width between the tips of the branches? If a main trunk is present, how long is it? What is its diameter? What are the length and diameter of the longest branch? Of the shortest? Compare several specimens with respect to the features mentioned above.

- c. Color.*—What is the color of *Spongilla*? Is it a color common to animals? Compare several specimens to note variations in the color of the entire body and in its various parts. Where is the prevailing color most intense? How do you explain this fact? Can you suggest any use for this particular color?

*d. Structure.*

1. The **body-substance** or "**flesh**."—With the unaided eye or with a hand-lens what can you make out with regard to the substance of which the body is composed—its color, consistence, etc.? Is the surface of the body smooth, or does it present elevations? If the latter, are there places where the elevations are more numerous than elsewhere? Note the arrangement, shape, and size of the openings or **pores** scattered over the surface of the body. What relations exist between the pores and the elevations, if any of the latter are found? Explain reasons for same.
2. The **microscopic structure**.—With fine forceps carefully tear off a small portion of the body-substance, mount it in a drop of water, and examine under the low power. Note the body-substance. Is it granular or homogeneous? If the former, of what do the granules consist? What is their color? Note the transparent, pointed bodies or **spicules** embedded in the body-substance. Do they have a definite arrangement? Are they numerous or few? Are they evenly scattered through the body? What is their shape? Do you find any parts corresponding to the fibres of the toilet sponge?

Put on the high power and look for isolated cells which have been separated from the fragment of the body. How many kinds of cell do you find? Are any of them amoeboid in shape? Any flagellate? Do they remind you of any *Protozoa* you have seen? Are the cells



closely joined together as in a membranous tissue, or do they readily separate from one another? What do they contain? Do you find that the cells bear any definite relation to the spicules? Examine some of the spicules more carefully, and note the dark line (cavity?) running through the middle. Run a drop of acetic acid carmine under the cover-glass. What changes take place in the cells? In the spicules? Can you find nuclei in the former? Mount a fresh preparation and run a drop of hydrochloric acid under the cover-glass. What effect has the acid on the spicules? If the spicules dissolve, forming bubbles, they are composed of carbonate of lime, the bubbles being carbon dioxide; if the spicules remain undissolved they are composed of flint, this substance and carbonate of lime being the materials of which the spicules of various sponges consist.

Look for the **statoblasts**, **amphidiscs**, or **gemmules**, yellowish toothed disks connected by a rod-shaped piece, which are formed in the autumn and serve to reproduce the sponge in the following spring.

#### PHYSIOLOGY

- a. *Movements*.—Can you tell from watching the living *Spongilla*, and noting especially its mode of attachment, whether or not it can move from place to place? How does it compare in this respect with the toilet sponge, judging entirely by what you have observed in the structure of the latter?

Can you decide whether or not the branches of *Spongilla* move? With a very fine bristle carefully touch the surface of the body-substance, several times if necessary, in the neighborhood of an osculum. Do you detect any change in the latter? Does it open wider or close? Can *Spongilla* feel?

Put a fragment of the body-substance of living *Spongilla* under the microscope, and note the motions of the amoeboid and flagellate cells.

*b. Ingestion.*—Sift some particles of finely powdered chalk, indigo, or carmine into the water containing living *Spongilla*, and look through the water towards the light to see whether the particles are drawn into the oscula or washed away from them? Can you from your own observations explain why the current of water flows in this direction? What, then, seems to be the function of the oscula?

*General Questions.*—Is there anything about the structure or mode of life of *Spongilla* which would lead you to suppose that it might be used as food or destroyed in other ways by water animals? If so, has it any means of protection against them? By what means may it be distributed through lakes and rivers?

**EXAMPLE 3.—Grantia (Sp.)**

*Material.*—Students living along the New England coast can obtain this sponge in the living condition, in which case some points in its physiology may be studied as indicated for *Spongilla*. It is especially valuable,

however, on account of the simplicity of its structure. The following study is based upon alcoholic material, which the student may prepare for himself or may obtain from sources mentioned in the introduction. Specimens of two kinds may be prepared, the first to retain the spicules, the second to be decalcified so that the tissues may be examined. The first kind of specimens may be dried, or may, while still living, be placed into seventy-five per cent. alcohol, where they should remain for a day, then they may be transferred to ninety per cent. alcohol and left until needed for examination. In each case the bulk of the alcohol should be several times that of the specimens. Decalcified specimens may be prepared by placing either living or alcoholic sponges into one per cent. to two per cent. solution of chromic acid for twenty-four to thirty-six hours, during which time the acid removes the spicules by dissolving them, but hardens and preserves the cellular tissues. The decalcified sponges are then to be passed through the various grades of alcohol, being left in the strongest until it is no longer discolored by the acid, embedded in celloidin or paraffine, and sectioned free-hand with the razor or, better, on the microtome.

Other material required: Delafield's hæmatoxylin, borax carmine, or acetic acid carmine, fifty per cent. glycerine or Canada balsam, watch-glass, test-tube, alcohol lamp, pipette, potash, compound microscope, and hydrochloric acid.

*Method of Examination.*—Study first, with or without the hand-lens, the entire specimen in the living state if obtainable; if not, alcoholic material may be used, the specimen being kept in a watch-glass or small dish containing fifty per cent. alcohol. Other specimens,

preferably alcoholic, as these are tougher, should be divided into halves longitudinally with the razor, and still others cut transversely. If the conveniences necessary for celloidin or paraffine embedding and the microtome are not at hand, fair sections may, after a little practice, be made with the razor from well-hardened alcoholic material. Such sections may be stained in Delafield's hæmatoxylin, borax carmine, or, if decalcified, in acetic acid carmine, and then mounted in water, fifty per cent. glycerine, or Canada balsam after passing through the preliminary treatment required by the different fluids.

Spicules may be isolated for examination free from the tissues, by placing specimens in a test-tube or watch-glass containing potash, boiling for a few minutes, during which the fleshy parts of the body dissolve, allowing the sediment to settle, pouring off the fluid, and rinsing the sediment several times with fresh water, being careful each time to allow the sediment to settle before pouring off the water. A drop of the sediment may then be taken out of the test-tube with a pipette and placed on the slide for examination.

#### MORPHOLOGY

With an entire specimen notice :

- a. Shape.*—What is the usual shape of a single sponge? Is it a symmetrical form? Can you distinguish an upper and a lower end? How? Is the sponge attached to anything, or is it free to move about? If the former, to what is it attached? Is its shape at all modified for this purpose? Do you find any decided variations from the general shape? Can you explain them? Are all of your specimens

single individuals, or do you find evidences of budding? If the latter, from what part of the parent sponge does the bud grow? Does it differ much from the parent in shape?

Make enlarged drawings of single and of budded specimens.

b. *Size*.—What are the length and diameter of your largest specimen? Of your smallest? How many different sizes of the bud do you find? Have you any specimens which, as regards size, are of commercial value?

c. *Color*.—What is the color of the living sponge? Of the alcoholic specimen? Compare with *Spongilla*. Has your specimen the same color as the object to which it is attached?

d. *Structure*.

1. The **osculum** or excurrent opening.—Where is it? Do you find more than one on an individual sponge? How does its diameter compare with that of the body? Notice the cluster of spicules around the osculum. How are they arranged? Do *any* of the buds have oscula? Does *each* have an osculum?

2. The **body-cavity, gastral cavity, or cloaca**.—In a specimen without buds, which has been halved lengthwise, note the body-cavity extending downward from the osculum. What is the shape of the cavity? How far into the mass of the sponge-body does it extend? Do you find any variations in its diameter? How does its diameter compare with the thickness of the body-wall? Do you find that the body-

cavity has branches? Make a longitudinal section through the middle of a budded specimen, and note whether a branch of the body-cavity of the parent extends into the bud.

3. The **body-wall**. — Examine the longitudinal sections with a low power. Note the spicules covering the surface of the sponge, also the whitish sponge flesh, and on the cut surface of the section the longitudinal (radial) canals or incurrent openings running through the wall from the surface toward the cloaca. Are these canals numerous? What is their shape as seen lengthwise and endwise? How are the outer ends of these canals guarded? Are the inner ends likewise protected? Why? Notice the mass of débris among the spicules which cover the surface of the body. In what ways do the flesh and spicules of the bud differ from those of the parent? Examine transverse sections of the sponge. Do you find that the body-wall projects into the body-cavity in such a way as to subdivide the latter into smaller cavities? Or is it a single continuous cavity?
4. The **spicules**. — Prepare some spicules as directed, and examine under the low power. How many shapes do you find? Draw each kind. Compare with the longitudinal section of the sponge, and note in what part of the body each kind of spicule is found. What is the shape of the spicules found on the surface of the sponge? Of those embedded in the flesh? Can you suggest any reasons for these shapes? Put a drop of hydrochloric acid upon some of the isolated spicules. What is the re-

sult? Compare with the same experiment on the spicules of *Spongilla*. What difference? Of what are the spicules of *Grantia* composed?

5. The **histological structure**.—Good results can usually be obtained only from material which has been stained—*e. g.*, in Delafield's hæmatoxylin, eosin, or borax carmine—embedded in celloidin or paraffine, and sectioned preferably on a microtome. If such sections can be obtained, the more minute structure of the sponge may be studied under the high power. It will then be seen that the sponge flesh or **syncytium**, which fills all the space among the radial canals and in which the spicules are embedded, is made up of granular protoplasm in which many nuclei are prominent. Sometimes the outlines of the constituent cells may be distinguished. The radial canals are lined with a layer of cells which form the **endoderm**. Possibly, in well-prepared specimens, each cell of the endoderm may be seen to have a single long cilium or flagellum. In places may be found dark-colored oval or spherical masses, **eggs** or **embryos**, lying in the syncytium just below the endoderm.

An excellent method of obtaining isolated sponge cells for microscopic examination is to put living sponges, as *Grantia*, *Chalinula*, etc., into a dish of sea water, quietly remove nearly all of the water with a pipette, then quickly pour over them a saturated solution of corrosive sublimate. After a moment the water in the neighborhood of the sponge, especially if carefully agitated, will become milky. Put some of this cloudy water on the

slide and examine with a high power. Note the great number of amœboid cells with their processes, of single and clustered flagellate cells, of spicules embedded in amœboid cells, of diatoms and other foreign bodies which wash out of the sponge body.

Run a drop of Delafield's hæmatoxylin under the cover-glass and note the distinctness with which the nuclei of various cells are brought out, also the collars and flagella of cells from the endoderm. Compare these cells with the ciliated cells examined on page 26.

Look for amœboid cells which have ingested diatoms, plant-spores, etc.



### **Fresh-water Polyp (*Hydra Sp.*)**

*Material.*—It is seldom that one is so situated as to have a supply of living hydras at hand whenever he wishes, as they are very unreliable animals in their habits; they may be found in great abundance one year and not at all the next. Very little can be given in the way of definite directions for obtaining living specimens, and, owing to the difficulty of killing them in the expanded state, those preserved in alcohol are usually so shrunken as seldom to be suitable for beginners to study. Perhaps the best method of obtaining material is to get from stagnant ponds or marshy lakes a good supply of submerged or floating water weeds, for example *Elodea Canadensis* and duck-weed (*Lemna*), put that gathered at different places into separate glass jars, with a label on each to indicate the locality whence the material came, and set the jars filled with clear, fresh water on a table near a window, but not exposed to the direct sunlight. In the course of a few hours carefully examine the lighted side of each jar for hydras. Having learned in this manner that specimens are to be found in a certain locality, further supplies of water weeds may be gathered. If the number of specimens found should not be sufficiently large to supply the class, work on this form may be delayed until additional specimens can be raised. This may easily be

done by keeping the jars exposed to diffused light in a warm room, and supplied with water plants and small fresh-water crustaceans, as *Daphnia*, *Cypris*, etc. It may be well to keep the jars covered to prevent evaporation and the access of dust. From time to time the jars may be set in the sunlight, in which case it is best to darken the lower one-third of each jar by wrapping it in a dark cloth or setting it in a close-fitting paste-board ring made from a hat-box. In this way over three hundred hydras were once raised during one winter in a jar which in the previous autumn was known to contain only about twenty specimens. Bits of raw meat, hand-lens, dissecting needles, bristle, wire, tumbler, dilute acetic acid, and compound microscope will also be required.

*Method of Examination.*—Specimens are very likely to be found on the lighted side of jars which have stood near a window for some hours. Without disturbing the jars in the least first examine the animals without and with a hand-lens, noting as many as possible of the characters given on the following pages; then, if the hydra is attached to the side of the jar, by means of a pipette suck the animal loose from its point of attachment, previously loosening its attachment with a camel's-hair brush, or, if fastened to a root of water plant, snip off the root with a pair of fine scissors, transfer with enough water to cover well to a watch-glass or a concave slide in which the specimen may again be examined with the lens. If light-colored, the animal may be made more plainly visible by placing the watch-glass on a dark background, as a black book or dark paper, or, if dark-colored, *vice versa*. Later, place the watch-glass upon the stage of the microscope, and

study both in direct and in reflected light with the low power.

#### MORPHOLOGY

- a. Size.*—Is the animal plainly visible? Do its length and breadth vary at different times? Is there any relation between the variations in the two dimensions? Does the size of the attached portion or **disk** vary?
- b. Shape.*—What is the general shape of the body? Does the body keep a definite shape? Is it symmetrical? Is the body flexible or rigid? Do you find any specimens whose bodies are plainly irregular in shape? Are these irregularities the same in position and size on different individuals? Do you find any specimens which appear to be colonies rather than single individuals? If so, of how many individuals does the colony consist? Are all the members of the colony equally well developed? At what points are their bodies united?
- c. Color.*—What is the general color of the body? Does it vary in different individuals? Is the body of the same individual evenly colored? Does its color vary at different times?
- d. Structure.*—Make out the following parts of the body:
1. The **disk** or **foot** (attached portion).—How much of the entire body does it form? How does it compare in diameter with the body proper? What is its outline? Is the body attached by any other part than by its base?

2. The **body proper**.—What is its shape? Does the shape change? Is its diameter uniform throughout? Compare the color of this part with that of the disk. Can you discover a cavity or **enteron** inside the body-wall? Note the **mouth** at the upper end (**hypostome**) of the body proper. What is the position of the mouth with regard to the tentacles? Endeavor to find a specimen lying with the mouth directed towards you, so as to see its shape and size.
3. The **tentacles**.—What is their position on the body? How are they arranged? How many can you find? Is their number invariable on different specimens? Are the tentacles of the same size? Do they ever vary individually or collectively in this respect? If both the brown and green species of *Hydra* are available, compare the tentacles as to number and variability in both forms.

Make drawings illustrating all of the structures studied thus far.

#### PHYSIOLOGY

##### a. *Movements.*

1. *Contractility*.—What various motions can you detect in the body proper? In the tentacles? Do either the tentacles or the body change in shape, size, or color as they move? Are the movements of the various parts at all uniform or rhythmic in character?
2. *Locomotion*.—Examine a jar of hydras, for some time if necessary, to see whether or not you can find any of them moving about. If not, with a pen or with a pencil which will mark on glass or with a fine camel's-hair brush put a

small dot on the surface of the glass jar so as to cover the point of attachment of each of several hydras. Leave the jar undisturbed for a time, then look to see if any of the animals have changed or are changing position. If not, quietly turn the jar a little, so that the light may strike it from a slightly different direction, and examine again after a time. Have any of the hydras moved? If so, in what direction? Do all move? Do they migrate at the same time? At the same rate? To the same distance from the original position? Indicate the new position by a new set of marks, as by circles or dashes, endeavoring to trace the path of each animal. Turn the jar again and repeat the examination. If the student has time and inclination, let him watch to see the manner in which the hydra moves from place to place. Does it use the foot alone? How rapid is its progression?

If the above experiment prove successful, what would you say has caused the hydra to change place?

The following method may also be tried to induce the hydras to move: With a fine thread suspend a small piece (about one-eighth of an inch square) of raw beef against the inside of the jar of water in which the hydras are kept. If possible, hang the meat in the midst of a group of the animals in such a position that the meat will be an inch or more distant from each hydra. Does the meat prove attractive? If so, which animals move first, those above, below, or at one side of the meat?

Lay a wire across the top of the jar, and from the wire suspend by means of a thread a second scrap of meat out in the water a distance of an inch or two from the side of the jar and near another group of hydras. Do any of the hydras get out to the meat? If so, how? Judging from these experiments, what methods of locomotion would you ascribe to the hydra?

- b. *Nutrition*.—Put a few scraps of raw beef about as large as the head of a pin into a watch-glass containing two or three hydras, and watch with the low power or with a hand-lens to see the behavior of the animals. In what way do they seize the meat? How is it conveyed to the mouth? How swallowed? With the hand-lens carefully examine a number of specimens attached to the inside of the glass jar to see whether or not you can find any which have caught any of the water fleas or other small crustaceans which were put in with the hydras. How does the hydra behave when any of the crustaceans come in contact with it? What apparently happens to the crustacean? Look for hydras which have swallowed small water animals, and note their position and condition in the body of the hydra.

c. *Nervous Properties*.

1. *Irritability*.—Is the hydra disturbed when irritated by coming in contact with a foreign body, as when gently touched once with a fine bristle? What does the animal do under such circumstances? What is its behavior upon being touched repeatedly?

2. *Influence of light (heliotropism).*—Put several hydras into a beaker or a tumbler, give them time to become fixed in position, mark on the glass the position of each individual, then cover the top and sides of the beaker with thick opaque paper or cloth. At one side make a hole about one-half inch in diameter through the paper to the glass, so as to admit the light. Set the beaker in a well-lighted place, but not in direct sunlight, and examine after a few hours' exposure. Have the hydras changed their positions? If so, what relation does their present position bear to that of the hole in the paper? Are hydras sensitive to a small amount of light?
3. *Co-ordination.*—Do the movements of the hydra seem to be made at random or for a purpose? Do any of your observations lead you to think that the animal performs intelligent actions?

*Microscopic Structure.*—Put on the high power and review all of the doubtful points of morphology and physiology, and in addition investigate the following topics: Is the stomach cavity or **enteron** continuous throughout the body proper? Can you discover an intestine? Does the enteron extend into the tentacles? Is there an opening in the foot? Can you find any opening, the **anus**, through which waste matter may leave the body? Study the structure of the body-wall, and note the two cell-layers, the outer or **ectoderm** and the inner or **entoderm**, and between the two the supporting layer or **mesogloea**. Does the ectoderm cover the entire surface of the

body, including the tentacles? How much of the thickness of the body-wall does this layer form? What are the shape, relative size, color, and arrangement of the cells in this layer? Examine both extended and contracted specimens with regard to the points last mentioned. Scattered among the ectoderm cells on the tentacles, notice the "thread cells" or **cnidoblasts**, in which lie highly refractive capsules, the **nematocysts**, each enclosing a spirally-coiled thread, and the outer end of which terminates in a sharp spine, the **cnidocil**. On what part of the tentacle are the nematocysts most abundant? Are they regularly arranged? Do you find any on the body proper? On the hypostome? How do they compare in shape and size with the ordinary ectodermal cells? Notice that, in addition to the two kinds of cells already studied, the ectoderm also contains among the bases of the larger cells small or **interstitial** cells. Compare these with the others.

Does the entoderm line the entire body-cavity? Does it extend into the tentacles? How does it compare with the ectoderm in thickness? Are its cells larger or smaller than those of the ectoderm? What is their shape? Color? To what is the color due? Can you see a circulation of food-particles in the body-cavity? In the tentacles? Can you detect the cause of this circulation? Do any of the entodermal cells bear cilia or flagella? What changes take place in the entoderm when the animal contracts? How thick is the supporting layer? Can you trace it throughout the body? Can you discover whether or not it is composed of cells?



Draw a portion of the body-wall, showing the arrangement of the cell-layers and the shape and position of the constituent cells.

Can you detect the presence of eyes or of any organs by which the hydra can perceive light? Do you find any nerves or muscles? Brain? Do you find organs of *any* kind inside the body? After the foregoing topics have been studied, run a drop of dilute acetic acid under the cover-glass. What change takes place in the position of the body and tentacles? What happens to the nematocysts? Notice the shape and length of the threads. Of what use to the animal can be this behavior of the nematocysts?

From a well-fed community of hydras select an individual which bears buds, and in addition to the topics studied before, investigate the following: Does the enteron of the parent extend into the bud? Do the cell-layers do the same? Do all of the buds have tentacles? Up to what point in their development do the buds remain attached to the parent? To what extent and for how long is the bud dependent upon the parent for food-supply? Do you find any buds which are just about to leave the parent? How do you distinguish them? What appear to be the first steps in the formation of a bud?

Make sketches showing the manner in which the bud is attached to the parent, buds in various stages of development, and the relation of the enteron and cell-layers of the bud to those of the parent.

Examine mature individuals for the presence of sexual glands (**testes** and **ovaries**), both of which may be found on the same animal, the former as small, knob-like swellings near the tentacles, the latter as larger spherical swellings farther down on the body. How many testes do you find on one individual? Is the

number constant? In which layer and from which cells do the testes develop? Compare the ovaries with the testes. Can you find the **ovum** itself? Press the cover-glass so as to crush the testes, and note the **spermatozoa**. What is their shape? Size? Are they motile or motionless?

Draw a specimen in which these organs are visible.

An excellent method of preparing isolated cells of the ectoderm and entoderm for study is to put a hydra into Müller's fluid for a day, then with fine dissecting needles carefully tease it to pieces in fifty per cent. glycerine, and examine under high power. Prepared sections of the body may be made according to the directions given in the revised edition of Huxley and Martin's "Practical Biology."

### **Campanularian Hydroid (*Campanularia* Sp.)**

*Material.*—The student should examine both living and alcoholic specimens. The former, however, can be obtained only by students who live not more than a day's journey from the coast. Pieces of rock-weed, various sea-weeds, eel-grass, bark, shells, etc., bearing specimens may be packed for shipment in a ventilated box with plenty of damp sea-weed. On arrival at their destination the specimens should be placed at once in carefully prepared artificial sea-water or in well-aerated natural sea-water, which may be shipped in casks. The hydroids may be kept alive long enough to give the student some idea of their appearance and motions.

The structure is best studied in preserved specimens, and these are made by plunging as quickly as possible, so as to avoid contraction of the tentacles, the living hydroids into Perenyi's fluid, and leaving them for three to four hours, or in Kleinenberg's picro-sulphuric acid for the same length of time, then placing them in seventy per cent. alcohol for about twelve hours, and in eighty-five per cent. and ninety-five per cent. alcohol, each for the same length of time. Some of the preserved material should be stained in hæmatoxylin, borax carmine, alcoholic carminic acid, etc., and mounted in Canada balsam. The rest may be left unstained and used for both macroscopic and microscopic examination.

The free-swimming medusoid forms are obtained by skimming the surface of the water on warm, still nights with a net of bolting-cloth (for full directions see Brooks's "Invertebrate Zoölogy"). The specimens thus caught may be killed in good condition by a short immersion in one-tenth per cent. osmic acid. Then transfer to a watch-glass of thirty per cent. alcohol, and finish the hardening in stronger grades made by adding to that in the watch-glass a few drops of ninety-five per cent. alcohol every few minutes.

*Method of Examination.*—Living specimens free from foreign bodies, algæ, mud, etc., may be examined in a watch-glass of sea-water. Study alcoholic material in a watch-glass of fifty per cent. alcohol. Specimens mounted in Canada balsam are, of course, "permanent mounts," and are ready for microscopic study at any time.

#### MORPHOLOGY

Examine a cluster of the animals in a watch-glass filled with fifty per cent. alcohol and note the plant-like aspect of the cluster. Note also the root-like attached end or **hydrorhiza**, the main stalk or **hydrocaulus** with its branches, and the position of the **zooids** or **hydranths** on the branches. Then examine both living and prepared specimens, first with a low, then with a high power.

*a. General Form and Structure.*—What is the general shape? How do you distinguish the base of the specimen? Is the cluster a colony of animals or a single animal? How are the branches arranged on the main stem? Are there any dif-

ferences among the buds or zoöids occupying the branches? How many kinds are distinguishable? Is the transparent covering or **perisarc** continuous over the entire cluster? Is the fleshy part or **cœnosarc** continuous?

- b. The **perisarc**.—Of what does it appear to be composed? What is its color? Does it vary in thickness in different parts of the cluster? Explain. Note the **annulations** at the bases of the branches. Do these annulations occur anywhere else? What is the usual number of annulations on a branch?
- c. The **cœnosarc**.—Where is it most plainly seen? Is it attached to the perisarc at all points? Is it everywhere covered by the perisarc? Note that it is composed of two distinct layers of cells, the **ectoderm** or outside layer, and the **entoderm** or inside layer. How do these compare in thickness? In color? How are they connected? Do you notice any differences between the cells composing each layer? Can you detect a third or **supporting** layer lying between the other two? How do you distinguish it from them? Are these layers traceable everywhere in the cœnosarc? Compare with *Hydra*. Notice that through the centre of the cœnosarc runs a tube or **body-cavity**. Can you trace this tube throughout the entire colony?
- d. The **zoöids**.—Endeavor to find the kinds mentioned below:
1. The *feeding zoöids* or *hydranths*.—What is their position in the cluster? Their shape? Are

they entirely covered by the perisarc? What is the shape of that portion (**hydrotheca**) of the perisarc which covers the body of the zoöid? Note the tentacles. How many has each hydranth? What is their shape? Of what are they composed? How are they arranged? Note the groups of **nematocysts** or "**lasso-cells**" on the tentacles. How are they arranged? Are the tentacles flexible or rigid? If living hydroids can be obtained isolate some of the nematocysts by crushing a tentacle under the cover-glass, and note their structure. Compare with *Hydra*. Examine also the mouth at the outer end, or **manubrium**, of the body proper. Compare several zoöids to see if the manubrium is flexible and the mouth distensible. What is its structure? Does it bear tentacles? Examine the body of the hydranth. What is its shape? Of what is it composed? What is the shape of the body or *digestive* cavity? Is the body-cavity a closed cavity? How far does it extend into the tentacles? Do you ever in preserved specimens find in this cavity small molluscs, crustaceans, etc., which have been captured for food? How does a hydranth compare in structure with a hydra?

Make an enlarged drawing of a single hydranth with its stalk, showing all of the structures visible.

2. The *reproductive zoöids*. — Position? Shape? Are they covered by the perisarc? Compare the shape of this portion (**gonangium**) of the perisarc with a hydrotheca. Do they have tentacles? How do these zoöids differ in internal

structure from the feeding zoöids? Do you find coenosarc, manubrium, digestive cavity, etc.? Examine the medusæ in the reproductive buds, and note their attachment to the central stalk or **blastostyle**, which morphologically represents a rudimentary hydranth. Of what is the blastostyle composed? How are the medusæ attached to it? How many medusæ in each gonangium? How do those in the same gonangium differ from one another? How do you account for these differences?

Draw a reproductive zoöid showing medusæ in various stages of development.

3. The *young zoöids*.—Note their position, shape, and structure. Look for them in different stages of development. Can you distinguish a young feeding zoöid from a young reproductive zoöid? Compare these young zoöids as to position in the colony, shape, structure, etc., with the buds on a hydra.

Draw young zoöids to show the various stages in their growth.

#### PHYSIOLOGY

If living hydroids are obtainable the following topics may be studied. Many of the questions, however, may be satisfactorily answered by a careful comparison of the shapes, positions, contents, etc., of dead specimens.

- a. *Movements*.—Does the colony as a whole sway back and forth on the hydrocaulus? Is the hydrocaulus flexible enough to be bent by currents in the water? Can you determine whether or not the colony can loosen the hydrorhiza from its

base of support and migrate to another place? Can the branches bend? What movements have the bodies of individual zoöids? Do the feeding zoöids have more or less mobility than the reproductive and young zoöids? Why? In what various ways do the tentacles move? Does the *cœnosarc* in the branches move at all?

*b. Feeding.*—Feed a colony with very small scraps of meat placed in a watch-glass of sea-water, or, better, watch the capture of small animals swimming about the colony, and compare in all respects with *Hydra*. How do the tentacles behave? Are the nematocysts used in this process? How is the food swallowed? Examine a living zoöid under the high power, and endeavor to see the circulation of currents of fluid bearing particles of food through the body-cavity. Can you detect on the cells of the entoderm the *cilia* whose motions cause these currents? Do the currents flow through the branches and into the reproductive and the young zoöids? Why? To what does this correspond in our own bodies?

*c. Sensation.*—While watching a colony in which the zoöids are expanded, gently disturb the water in the watch-glass. Are the zoöids disturbed? How do you tell? Carefully touch a single expanded zoöid with a fine bristle. What does the zoöid do? Are the neighboring zoöids which were not touched disturbed? If so, what property does this show the colony to possess?

*General Questions.*—Do you find any distinction be-



tween "body-cavity," in which various internal organs might be contained, and the "digestive cavity" in a zoöid? If not, would you consider these two cavities to be one and the same as regards these hydroids? Compare with *Hydra*. What means of offence and of defence have these hydroids? Of distribution? Which do you regard as the higher animal, a campanularian hydroid or a hydra? Why? How much of the colony do you regard as an individual animal? Why?

Compare with this organism any other hydroids that may be available, also the sea-anemone (*Metridium*).

### **Starfish (*Asterias Sp.*)**

*Material.*—Living specimens may be found almost anywhere along the sea-shore adhering to rocks, timbers of wharves, etc. It is best to collect them at low tide, as then they are usually near the surface or possibly above it; otherwise a dipping-net may be needed to reach them. As the starfish lives only in salt water, it may be difficult for students living far from the sea-shore to procure living animals; however, if packed in an abundance of wet sea-weed and kept cool, living starfishes may be sent to inland schools if not more than ten to twelve hours' ride from the coast. On arrival they should at once be transferred to sea-water, which may be sent in casks from the coast, or to artificial sea-water, where they may live for several hours, and thus give the student an opportunity to study their habits. The water should be kept well aerated. This may easily be done by repeatedly pouring from a height dipperfuls of the water into the vessel containing the starfishes. Specimens for dissection may be prepared in the following manner: The living animals should be dropped into seventy per cent. to ninety per cent. alcohol immediately after being taken out of the sea-water. The alcohol kills the animal almost immediately, and, as the rays retain the various positions which they had just before the death of the animal, it is well to select a series of specimens which have the rays bent and curved in dif-

ferent directions, in order that the inland student may see even in the preserved specimens the wonderful flexibility of the body and rays of the starfish. Care must be taken not to put too many specimens into the vessel of alcohol, for the water in the body-cavity and tissues of each animal weakens the alcohol a certain amount. With ninety per cent. alcohol the bulk of the specimens should be not more than one-third of the bulk of the alcohol used. After the specimens have lain in strong alcohol for an hour or two, insert the needle of a hypodermic syringe into the roof of each ray, near the tip, and fill the cavity of the ray with alcohol. This preserves the internal organs in good condition, keeps the ray distended in its natural shape, and the hole made by the needle does not interfere with dissection; besides, the hole, being very small, becomes plugged up immediately after the withdrawal of the needle, and thus the alcohol is kept from oozing out, as it would through a larger opening. After being in the strong alcohol for two or three days, the specimens may be kept indefinitely in seventy per cent. to ninety-five per cent. alcohol. For the study of the hard parts alone, however, it is well to use dried specimens. These are prepared by taking some of the alcoholic specimens treated as above, and laying them for a day or two in the hot sun or in an oven. If dried in the open air, they should not be left out of doors overnight nor allowed to become damp. For the study of the structure and arrangement of the individual portions of the skeleton alcoholic or dried specimens may be soaked for a few days in a ten per cent. solution of potash. As soon as the soft parts commence to macerate, the specimens may be carefully brushed with a ragged tooth-brush in order to remove the skin and flesh. If soaked too long, the skeleton will drop to

pieces. When thoroughly cleaned, rinse the skeleton in fresh water and put it into strong alcohol for four or five hours, then dry in the open air or over a stove.

For the study of the water-vascular system, injected specimens should be prepared. A very convenient method is to kill the starfish in fresh water; after the animal is dead warm the water up to the melting-point of gelatin, cut off the tip of one of the rays, insert the tip of a fine-pointed syringe into the end of the radial water-tube, and with a slow but firm pressure fill the water-vascular system with a carmine or Prussian-blue injecting mass. Specimens thus prepared may then be hardened in alcohol, where they will keep indefinitely.

For the microscopical examination of cross-sections of the rays and disk, small specimens one-half inch to an inch in width should be provided. They may be found on rock-weed, eel-grass, the surface of the mud, etc., in quiet pools along the sea-shore during the summer months. They should be quickly picked off the surface to which they are adhering and instantly dropped into strong alcohol (ninety-five per cent. to one hundred per cent.) and left for four to six hours. The alcohol causes instant death, and the ambulacral feet and tentacles remain expanded. The specimens may then be placed in one-half per cent. to one per cent. chromic acid for about twenty-four hours, or at least until no more bubbles arise, showing that the acid has dissolved all or nearly all of the calcareous part of the body, and left it in condition to be cut with a sharp knife. The starfish is then placed in seventy-five per cent. alcohol for a day, then into ninety per cent. for another day or until wanted for examination. Specimens may be embedded in paraffin, or in celloidin, and cut on the microtome.

They may be stained in borax carmine either before or after cutting, or left unstained as desired.

The larval forms of the starfish may be obtained along the New England coast from June to September, by skimming, or they may be raised from the eggs. Very small starfishes, suitable for study with the microscope, may be found attached to eel-grass during the summer. If a number of starfishes be caught during the time mentioned and be kept for a few hours in a pail of sea-water, some of them will probably be found to be discharging eggs or sperm. The former may be distinguished by their pinkish color; the latter is white. About a teaspoonful of the eggs may be thoroughly mixed with a few drops of sperm in a tumblerful of sea-water and set in a cool place. By means of a pipette the water should be changed three or four times a day and aerated frequently. Some of the eggs may be taken out from time to time to watch the process of segmentation, or this process may be studied in eggs fertilized on the slide. Eggs in various stages of segmentation may be taken from the tumbler at intervals, and preserved for further study after treatment as follows: Place the eggs for ten or fifteen minutes in Kleinenberg's picro-sulphuric acid (undiluted); transfer to thirty-five per cent., then to fifty per cent., alcohol each for an hour; then place them in seventy per cent. alcohol, and change the latter as often as it becomes discolored. Such specimens may be stained in Delafield's hæmatoxylin. Spermatozoa, and eggs showing the formation of the polar globules, seldom preserve satisfactorily. Another method of obtaining eggs and sperm is to cut open the body, remove the egg and sperm-glands, and chop up the glands together in a watch-glass of water, or chop them separately and mix them after-

wards. The shreds of tissue should be removed, as their decay will pollute the water. Unfertilized eggs as well as those in various stages of development may, however, be preserved as alcoholic specimens for inland students to study.

A large scalpel or cartilage knife, a small scalpel, fine forceps, bristles, fine scissors, ten per cent. hydrochloric acid, test-tube or watch-glass, hand-lens, compound microscope, borax carmine, and Delafield's hæmatoxylin will also be needed.

*Method of Examination.*—Fresh specimens should be placed in a large vegetable dish of sea-water, which should be frequently renewed. Alcoholic material may be examined in a dish or dissecting-pan containing enough fifty per cent. alcohol to cover the specimen.

#### MORPHOLOGY

*External anatomy.*—With a fresh or alcoholic specimen study

*a. Shape.*—Is the shape that of a perfect star? Does it have a definite number of rays? Are all of the rays on a normal specimen of the same shape, size, etc.? What differences in shape between the under or oral and upper or aboral sides? How do you account for the extreme differences in the size of the body and rays of certain specimens? For the differences in the number of rays? Does the animal have a head? What significance has the common name? Is the animal a fish? Is the body bilaterally or radially symmetrical?

Draw both oral and aboral surfaces. Make outline

drawings of several specimens to show the normal shape and abnormal variations.

*b. Size.*—What is the size of an ordinary specimen? Can you tell anything about the age by the size?

*c. Color.*—What is the natural color? Are all the living specimens colored alike? Is the color the same all over the body? Are there any color markings on the body? What changes of color take place when the specimen is dried?

*d. Structure.*—The central part of the body is called

1. The **disk**.—What is its shape? How does its oral differ from its aboral side?

On one side of the disk find a circular plate,

2. The **madreporic body**.—Position? Shape? Size? Color? Structure?

The two rays which touch the madreporic body form

3. The **bivium**.—What differences between these rays?

The remaining three rays form

4. The **trivium**.—Compare with 3.

The ray opposite the madreporic body is called

5. The **anterior ray**.—Compare in shape, size, and structure with the other rays.

6. The **spines** (in studying these a dried specimen should be used for comparison).—On what part of the body are they found? Are there any traces of definite arrangement? How many kinds are there? What is their shape?

Size? Structure? Are they fixed or movable? If the latter, what kind of joint have they? Do you find any spines which seem to be specially adapted to certain purposes? Notice the five clusters of spines around the mouth, forming the **mouth-papillæ**.

Draw one of each kind of spine seen lengthwise.

7. The **skin**.—Does it cover all parts of the body? Examine its texture. What is its color? Do you find any variations in thickness? Is it loosely or closely attached to the hard parts of the body? With fine forceps remove a small piece of skin from the body of a living starfish and examine in a drop of sea-water with the high power. Note the **columnar ciliated epithelium**.

Among the bases of the spines find

8. The **aboral tentacles**.—How are they arranged on the body? How do they compare in shape, size, and structure with the ambulacral feet? Is their surface ciliated?
9. The **mouth**.—What is its position? Shape? Size? Are there any teeth? Lips?  
Around the mouth-opening find a membrane,
10. The **peristome**.—Shape? Structure? Color? Is it flexible? Does it bear any structures?  
On the oral side of the rays look for
11. The **ambulacral grooves**.—What is their position on the ray? Shape? How are they formed? What is their relation to the mouth? How far do they extend? Where is the deepest part of the groove? Where the shallowest?



In the grooves are

12. The **ambulacral feet**.—How are they arranged in the groove? What is their shape? Size? Do you find any variations in shape or size? Color? Structure? Are they found anywhere else than in the grooves? Pull off a foot and insert a fine bristle into the torn end. Notice that the foot is tubular. Examine one of the feet under the low power of the microscope, and note at the extreme end the thickened ring with the creased central membrane forming a sucking disk; also the circular ridges in the walls of the tube, the groups of circular muscles. Can you determine whether any or all of the feet are connected? Sketch a longitudinal section of an ambulacral foot magnified. Make an enlarged drawing of the sucking disk to show its structure.

Lying just above a membrane stretched across the roof of the groove find

13. The **radial water-tube**.—How far does it extend? Pass a fine guarded bristle into its cavity and trace the course of the tube. Examine injected specimens to see how the radial water-tubes are connected with one another. Along with the water-tube run a "blood-vessel" and a nerve so intimately connected with the tube as to be almost indistinguishable except in trans-sections of the ray prepared for microscopic examination.

Make a diagram of a cross section of a ray. At the ends of the rays look for

14. The **eyes**.—Position? Shape? Size? Color? What is the arrangement of the spines around the eye?

On the bases of the spines find

15. The **pedicellariæ**.—What is their exact position with regard to the spines? What is their shape? Size? Are they branched? Put one under the low power and examine the structure.

Draw.

*The Skeleton*.—In a well-cleaned specimen prepared as directed notice

- a. *General Structure*.—Of what does the skeleton consist? How are the parts or **ossicles** arranged? Do you find any differences in the arrangement of the parts on the oral and aboral surfaces? To what are the spines attached?

With a sharp knife cut off one of the rays at its widest part, and examine its oral surface.

Forming the roof of the groove find

- b. The **ambulacral ossicles**.—How are they arranged? What is their shape? In what direction do they run? Do they bear spines? Note the openings or **ambulacral pores**, through which the ambulacral feet pass. Are these openings perforations in the ossicles? If not, how are they formed? Are any two adjacent ambulacral ossicles exactly alike in structure?

Lying immediately outside the above is a single row of

- c. The **inter-ambulacral ossicles**.—What is their shape? Do they bear spines? If so, are these spines similar to those on the aboral surface of the ray?

Next outside the inter-ambulacral ossicles come

- d. The “**cross-shaped**” **ossicles**.—With what do they connect? What sort of spines do they bear? Do these ossicles consist of a single piece? Do you find any openings through them?

Cut along each side of the ray and remove the aboral portion, and notice in the middle of the floor of each ray

- e. The **vertebral ridge**.—How is it formed? Why called “vertebral”?

Remove the top of the disk and note

- f. The **mouth-opening**.—What is its shape? How formed? How does it compare in size and shape with the mouth itself?

- g. The **inter-radial partitions**.—How many are there? What position have these with reference to the mouth-opening? Of what are they formed? Look on each side of each partition for a small opening, the inner end of the **reproductive orifice**. Push a fine-pointed bristle into the orifice, and try to find the outlet of the tube.

- h. The **chemical composition of the ossicles**.—Drop a few of the ossicles into ten per cent. hydrochloric acid in a test-tube or watch-glass. The formation of bubbles in the fluid shows that the ossicles contain carbonate of lime. Compare with the spicules of *Spongilla* and *Grantha*.

*Internal Anatomy*.—Either fresh or alcoholic specimens may be used. The organs of the latter, though somewhat changed in color, possess the advantage of being toughened, and hence are less easily torn. With a pair of strong scissors make a transverse cut through the roof of the rays of the trivium, near the tip of each.

Extend the cut along the sides of each ray to the disk, in such a manner as to free the roof of the rays and disk from the lower part. Be careful not to injure the internal organs with the tips of the scissors. Bend back the cut portions and notice:

*a.* The **body-cavity, coelom, or peri-visceral cavity.**

—What is its shape? How is it formed? What does it contain? Is it single or divided into compartments? Note the smooth, glistening membrane lining the cavity. Examine microscopically a drop of fluid from the body-cavity of a living starfish.

*A.—The Digestive System.*

*a.* The **hepatic coeca** or "**liver**" (physiologically, a **pancreas**).—How many in each ray? Do they entirely fill the ray? How are they arranged? To what are they attached? How held in place? What is their color? Structure? With what do they communicate? By what means?

Attaching the hepatic coeca to the roof of the ray is

*b.* The **mesentery**.—What is its position? How many in each ray? Texture? Color? Notice that the mesentery is continuous with the membrane which lines the body-cavity. Try to prove that the membrane consists of a double fold.

*c.* The **stomach**.—What part of the body-cavity does it occupy? Note that it is divided into a pouched or **cardiac** portion and a pentagonal or **pyloric** portion. Study the arrangement, shape, size, and structure of the pouches. Examine the mem-

brane of which they are composed. Are the pouches distensible? To what are they attached? How does the cardiac compare in size with the pyloric portion? Do you find any ingested food in the cardiac portion? If so, of what does it consist? Do you find any structures which could serve to grind hard food? Do you find any specimens in which a part of the cardiac portion protrudes through the mouth? Look for the **retractor muscles** of the cardiac pouches. How many are there? What is their shape? Length? To what are they attached? Look for the **protractor muscles**. Compare them with the retractors. What is the position of the pyloric with reference to the cardiac portion? Do you notice any difference in the character of its wall? Notice the very short passage, **oesophagus**, leading from the mouth to the stomach. The shape of the various portions of the stomach may be well seen by proceeding as follows: Remove the roofs from all the rays, but leave the roof of the disk untouched. Separate the latter along the inter-radial partitions, divide the ducts of the hepatic cœca, remove the cœca from the body, turn the latter upside down, and pour water into the mouth. The water will distend the stomach and show its shape.

Near the centre of the aboral side of the pyloric portion of the stomach find

*d.* The **intestine**.—What is its shape? Length? Look for the opening, **anus**, to the exterior.

Near the intestine look for a brownish sacculated organ,

- e. The **respiratory tree**. — Position? Shape? Attachments?

Draw the digestive system alone.

*B.—The Reproductive System.*

At base of ray find the sexual glands, the **ovaries** pinkish, and the **testes** yellowish-white.

- a. The **reproductive organs**. — How many in each ray? Do you find both ovaries and testes in the same animal? Compare them as regards arrangement, shape, size, color, structure, etc., with the hepatic cœca. Where do they open? If possible, compare specimens collected in summer with others taken in the spring or autumn, and note the extreme differences in the size and color of these organs.

Draw the reproductive system in an outlined body.

*C.—The Water-vascular System.*

If obtainable, examine specimens in which the water-vascular system has been injected. Injected specimens may be prepared as directed. The madreporic body, ambulacral feet, and the radial water-tubes, all of which form part of the system, have previously been examined.

On each side of the vertebral ridge find

- a. The **ampullæ** or **water-sacs**. — What is their exact position in the ray? Shape? Size? Structure? Tear one open and note that it is hollow. Is its wall distensible? Lay a slender pencil in the ambulacral groove, so as to compress the ambulacral feet, and notice the effect upon the ampullæ. Is there communication between the ampullæ and the feet? How does the number of

ampullæ compare with the number of feet in the same way.

Cut all of the retractor and protractor muscles, cut the stomach across just above the peristome, and remove all of the digestive organs.

Running down under the madreporic plate find

- b. The **stone-canal**.—What is its position? Shape? Size? Pinch it lightly between the fine forceps. What is the nature of its wall? What does the canal connect? Does its end cover the entire lower surface of the madreporic plate?

Remove the peristome, and find lying within the mouth pentagon

- c. The **circum-oral water-tube**.—Does it lie without or within the body-cavity? What is its shape? Situated upon the circum-oral tube look for

- d. The **Polian vesicles**.—How many are there? What is their relation to the bases of the rays?

Extending horizontally into the cavity surrounding the oesophagus find

- e. The **racemose vesicles**.—How do they compare in position and size with the Polian vesicles? How many are there? How do you account for this number?

Make a drawing of the water-vascular system in an outlined body.

#### *D.—The Circulatory System.*

Enclosing the stone canal find a tube,

- a. The **pericardium**.—To what is it attached? What is the nature of its wall?

Lying within the pericardium look for

b. **The heart.**—How does it compare in size with the stone canal? Remove the heart from the body and examine under the low power of the microscope. What is the structure of the heart?

By injecting into the pericardium with a fine syringe a colored fluid, such as water containing carmine or indigo, the course of some of the **peri-hæmal tubes** which surround the true blood-vessels may be made plain. It will then be seen that there is (1) a **circum-oral peri-hæmal tube** surrounding the mouth just below the circum-oral water-tube; (2) a **radial peri-hæmal tube** running from (1) to the tip of each ray, likewise just below the radial water-tube; (3) a **circum-anal peri-hæmal tube**, which is on the inside of the aboral surface of the disk, and is larger than the peri-hæmal tube around the mouth. Its branches run to the aboral end of the pericardium, the stomach, the hepatic cœca, and the reproductive organs.

Make a diagram of the circulatory system.

*E.—The Respiratory System.*

Remove a piece (about one-half inch square) of the aboral wall of a ray, and carefully examine under water the depressions on its inner surface. Look for small openings in these depressions, and try to see if these openings bear any relation to the aboral tentacles on the outside of the ray. If necessary, use a fine bristle. Try to pass the bristle through one of the openings to see if the latter communicate with the tentacles. Carefully peel off the lining membrane. What effect has this operation on the tentacles? Notice that a tough membrane containing the ossicles remains. In the same manner remove the covering membrane on the outside.



What becomes of the tentacles? What lies below this outer membrane? Putting together the facts just learned, what can you say is the structure of an aboral tentacle? What is the structure of the membrane containing the ossicles? Does this membrane extend down between the ossicles, or does it merely cover their upper and under surfaces?

*F.—The Nervous System.*

With a lens carefully examine the lower surface of the circum-oral water-tube for a thickened ridge, the **circum-oral nerve-ring**, running around its outer surface. Running from this ring just below, *i. e.*, outside, the radial water-tube in each ray is a **radial nerve** which extends to the tips of the ray and ends at the eye. The relation of this nerve to the neighboring parts is much better seen in cross-sections of the ray prepared for microscopic examination.

PHYSIOLOGY

Nearly all of the following work must necessarily be done on the living animals. They should be studied among their natural surroundings whenever possible, otherwise in an aquarium well supplied with an abundance of running sea-water.

Experiments requiring the removal of portions of the body of the living animal probably cause little if any pain, since the starfish frequently parts with one or more of its rays voluntarily.

*A. Movements.*—What sort of motion has the starfish? How rapidly can it move? What are its organs

of motion? Can it swim? Does it always move with the same ray ahead? Study the movements of a single ambulacral foot. Are all of the feet used for progression? Are the rays flexible? If so, in what directions do they bend? Turn a specimen over on its aboral surface. Through what motions does it go in righting itself? Does the starfish lose the power of locomotion when removed from the water? Can it bend its rays under such circumstances?

*B. Nutrition.*—Look along the wharves at low tide for clusters of mussels on which starfishes may be found. Examine such a starfish to see if the cardiac portion of the stomach is not protruding and partially enwrapping a mussel. See if you can find that the stomach or disk moves at all. Quickly pick the starfish off the mussel and watch the withdrawal of the stomach into the body. How soon does it take place? In studying the internal anatomy, did you find any structures which could bring about this movement of the cardiac pouches? Do you find any specimens which though not feeding have the stomach protruding? Examine the stomachs of a number of fresh or alcoholic specimens for the shells of small molluscs. Do these shells bear any evidence of having been broken to pieces in the body of the starfish? Judging from the structure of the mouth-parts and of the stomach, what kind of food is the animal fitted to eat? Starfishes kept in an aquarium may sometimes be found feeding on other small animals kept in the same tank.

*C. Nervous Properties.*

- a. Touch.*—Does the starfish seem to feel objects with which it comes in contact as it moves about? Touch one of the feet with a pencil point or a bristle. Is the foot sensitive? In like manner touch one of the aboral tentacles. What happens? With a hand-lens find a large pedicellaria and touch the end of it with a fine bristle. What does the pedicellaria do? Do you find any organs which appear to be specialized as organs of touch? If so, where are they and from what have they been modified?
- b. Sight.*—Put a number of vigorous starfishes into a tank of sea-water and allow them to disperse at will. Note their positions, then cover the tank with thick cloth to exclude the light, leaving only a small aperture, two or three inches in diameter, at one end for a small amount of light to enter. After a time, fifteen minutes to several hours, look into the tank to see if the animals have changed their positions? Are they attracted by the light? How do you tell? From several starfishes carefully remove the eyes, noticing particularly the behavior of the surrounding spines, and put the animals into a tank prepared as above. Do they now pay any attention to the light? Have you any reasons for thinking that these animals can perceive small objects?
- c. Hearing, taste, and smell.*—Do any of your observations lead you to suppose that the starfish possesses these powers?
- d. Co-ordination.*—Examine a moving starfish and

note that the various rays and the multitude of ambulacral feet *all* move in such a way as to attain a common object. Study also the co-ordinated movements by which a starfish rights itself after having been overturned. Where does the righting movement begin? Does it ever begin on opposite sides of the body at the same time? Overturn the same animal repeatedly and see if the righting movements always begin on the same side of the body. With a pair of scissors remove one or more of the rays from the body of a vigorous starfish and put the parts back into the water. Does this treatment kill the starfish? Have the detached rays or the body lost their powers of motion, sensation, co-ordination, etc.? What is the direction of the motion of a detached ray? Of the body with the remaining rays? How do you explain this? Find a specimen which is progressing in a definite direction, and prick the skin on that side of the body towards the point to which the animal is going. Does the animal change its course?

#### *D. Reproduction.*

*I. Regeneration of lost portions of the body.*—With a pair of scissors or a sharp knife remove one of the rays close to the disk. Place the parts back into the water and study their behavior. What takes place at that part of the disk where the ray was removed? Watch the mutilated parts from day to day to see what changes are going on at the point of injury. How long before the opening into the body-cavity caused by the removal of the

ray is closed? How long before you can find evidences that a new ray is being formed to replace the one removed? Does this new ray ever reach the size of the others? If so, in how long a time? How many rays may be removed and still be replaced by new ones? Do the removed rays ever form a new disk, etc.? Try handling out of water large starfishes and see if they will voluntarily drop their rays. If so, at what point does the ray become detached? Is this property of any use to the animal?

Many of the above questions may be decided by a study of a collection of specimens of imperfect and mutilated starfishes.

## *II. Sexual reproduction.*

### *a. The structure of the sexual cells.*

1. The **spermatozoon**. — With a pipette collect some of the sperm which a mature male may be found shedding into the water, or remove the testes and with sharp scissors cut them to pieces in a watch-glass of sea-water. Put a drop of the sperm under the high power of the microscope, propping up the cover-glass with bits of wax or paper, and note the spermatozoa. Are they abundant or few? What sort of motions do they exhibit? Study their structure and endeavor to make out the **head**—its shape and relative size; and the **tail**—its shape and movements. Can you discover a cell-wall? Endeavor to stain the spermatozoa with hæmatoxylin. What part becomes most deeply stained? Is this part a nucleus?

Draw a spermatozoon.

2. The **ovum**.—Collect some ova in the manner described for spermatozoa. Put them under the low power and note their shape, size, color, etc. Is an ovum large enough to be seen without the microscope? Put on the high power and study their structure. Can you, with or without reagents, make out a cell-wall, protoplasm, and nucleus? Does the egg sink to the bottom or does it float? Compare ovum and spermatozoon as regards shape, size, color, structure, motions etc. What reasons can you suggest for the differences?

Draw an unfertilized, *i. e.*, ovarian, ovum.

*b. Preparation of the ovum for fertilization.*

1. *The formation of the polar globules or "direction-cells."*—Examine a number of unfertilized living ova to see if some cannot be found which are pushing out a small protuberance at one margin of the yolk (*protoplasm*), just under the membrane or *cell-wall*. This prominence increases in size, and finally becomes divided off from the surface of the yolk as the "first polar globule." Soon afterwards a second is formed in the same manner, and the two come to lie side by side under the egg-membrane. Each globule contains a portion of the nucleus of the ovum. Stain some of the ova with carmine or hæmatoxylin to prove this last statement. The process of the formation of the polar globules is known as the "maturation of the ovum";\*

\* A process somewhat similar to this has been seen to take place in the spermatozoa of certain animals.

in this manner the ovum becomes fitted for the reception of the sperm-cell (**fertilization**). How do the polar globules compare in size with the ovum? Do you ever find that more or fewer than two are formed? How long does it take the first one to form? The second? Does the second form in the same place as the first? Always? Can you suggest any reasons why the polar globules are formed?

Draw the different stages of formation of the polar bodies.

*c. Fertilization.*

1. *The union of the sexual cells.*—Mix together on the slide a drop of water containing a few eggs and one containing spermatozoa, put on the cover-glass supported by bits of wax, and examine under the high power. How do the spermatozoa behave? How the ova? Note that the most of the sperm-cells collect around the eggs. Can you see the entrance of the sperm-cell into the egg? Does it take place at any particular point? How many sperm-cells enter the same egg? Notice that a short time after the sperm and ova are mixed a clear area appears between the wall and the contents of certain ova. These ova have been fertilized, although the entrance of the spermatozoon may have escaped observation.

*d. The consequences of fertilization.*

1. *The first division or segmentation of the ovum.*—A few minutes after the sperm and eggs have been mixed some of the latter will show a notch beginning to form at one margin of the

yolk. This is the first external indication of the division of the egg. What relation has the position of this notch to that of the "direction-cells"? Can you suggest a reason why these are called "direction-cells"? Watch the notch as it deepens into a furrow. In what direction and how far does the furrow extend? How long before it divides the yolk into two distinct parts, blastomeres? What is the shape of each of these? Can you distinguish a nucleus in each part? Try reagents if necessary. Does this division have any effect upon the membrane? Do all of the eggs with which you have mixed sperm begin to divide at the same time, and does the process go on with the same rapidity in all?

Draw.

If reagents were applied while watching the first segmentation, fresh specimens must be taken for the following stage:

2. *The second division.*—How long before the second division begins? What is the first indication that a second furrow is beginning to form? What is its direction with respect to the first? How long before the egg is completely divided by this furrow? How many cells are there now inside the egg membrane?

Draw different stages of the second segmentation.

3. *The third division.*—Compare this with the first two as regards the various features mentioned.

Draw.

4. *The blastula.*—After a few hours, during which successive divisions take place, the egg will be seen to consist of a solid spherical mass of cells



which after a time will push out from the centre of the sphere, thus forming a hollow sphere, the cavity now enclosed by the layer of cells being known as the **segmentation-cavity**. When the egg has reached this stage of development it may require careful focussing to show that the sphere is really hollow. Does the membrane still surround the egg? Has the egg increased in size? Can you detect any movement of the egg as a whole or of the sphere inside the egg membrane?

Draw a blastula as seen from the surface and in optical section, *i. e.*, as though one-half the sphere were removed, leaving the cavity visible.

5. The **gastrula**. — At the end of about twenty-four hours after fertilization, or perhaps sooner, it will be noticed that at a certain point the surface of the blastula becomes indented as though being pushed in (**invagination**). The depression increases, and the invaginated mass of cells encroaches upon the segmentation cavity. The depression is the primitive mouth or digestive cavity of the embryo or **gastrula**, which now consists of two layers of cells, the original outer layer, or **ectoderm**, and the layer of invaginated cells, or **entoderm**. Between these layers lies the segmentation cavity, while the entoderm lines the **digestive cavity**. In this stage the cells of the ectoderm will be seen to be covered with **cilia**, by means of which the embryo rotates within the egg-membrane. The membrane soon breaks, and the embryo swims about freely in the water. Before this happens it may be noticed that the

entoderm cells are giving off **amoeboid cells**, which move about in the segmentation cavity, and finally form a third layer, or **mesoderm**, between the other two.

Draw a gastrula.

6. The *later larval stages*.—If material be obtainable, the student can trace the change of the gastrula into the Bipinnaria stage, and from this the development of the young starfish, noting especially the extreme changes of form and structure, and drawing each stage.

Use the sand-star (*Ophiopholis*), sea-urchin (*Echinus* or *Arbacia*), sand-cake (*Echinarachnius*), and sea-cucumber (*Pentacta*). The examination of the skeletons of the first three is especially instructive, and a very valuable and interesting study may be made of the manner in which the same fundamental plan of structure is modified in various ways in these different organisms.

### **Earthworm (*Lumbricus Sp.*)**

*Material.*—Both living and alcoholic material should be at hand. The first may be obtained almost anywhere in firm, damp soil during the warm months of the year, especially from June to September, during which time the worms are breeding, and on warm nights are very likely to be found lying on the surface of the soil, with the most of the body outside the burrow. At this time a large number of fine specimens may frequently be caught by quietly examining the ground in a garden or lawn. It is necessary to step carefully, as the slightest jar disturbs the animals, and they immediately withdraw into their burrows. Larger and more perfect specimens may usually be caught in this way than by digging. A number, at least five or six for each student, should be obtained, and those which are to be preserved in alcohol should first be put into a basin of water and the dirt rinsed off their bodies, then they should be put for twelve hours into three to five times their bulk of fifty per cent. alcohol in a flat dish, in which they may be laid out straight. Transfer to seventy-five per cent. alcohol for a day, and then to strong alcohol for preservation. In winter, living worms may be found in the soil or under flower-pots in greenhouses. Specimens may be kept alive for study in the winter by putting them into large, well-drained flower-pots filled with fairly stiff soil containing a few dead leaves and covered with a sod. The

flower-pots should stand in a cool, light place, as in a cellar window, and should be watered sufficiently to keep the soil damp, but not saturated. From time to time small pieces of cabbage and of lettuce-leaves may be placed upon the sod for the worms to feed upon. Other material needed includes a dissecting-pan, fifty per cent. alcohol, hand-lens, fine forceps, fine scissors, magenta, acetic acid carmine, muriatic (hydrochloric) acid, a rough, unplaned board, a sheet of sand-paper, pipette, and compound microscope.

*Method of Examination.*—Living worms may be placed on a dissecting-tray or in a dissecting-pan for examination, and their bodies must be moistened with water from time to time. To study its method of burrowing, place the worm on the surface of moistened and fairly compact soil in a flower-pot. The course of the burrow may afterwards be traced by carefully picking away the soil, beginning at the opening.

Preserved specimens are best for the study of the morphology of the animal, and should be examined in a dissecting-pan containing enough fifty per cent. alcohol to cover the body of the worm. If the preserved specimens are too rigid when first taken out of the alcohol, they may be soaked in water for an hour or so until flexible.

#### MORPHOLOGY

##### *External Characters.*

- a. *General shape.*—What is it? Does it vary in different parts of the body? Can you distinguish an anterior and a posterior end? How? Is there a "head"? Can you distinguish a dorsal and a ventral surface? How? A right and a left

side? How? What is the length of the specimen examined? Where is the circumference of the body greatest? Where least? What is the shape of a cross-section of the body made through the largest part?

Make an outline drawing, twice natural size, of the worm seen from above, and of the cross-section.

*b. Color.*—What is the general color of the body? What differences in different regions? Can you give any reasons for these differences?

*c. General structure.*—Into how many distinct regions may the body be divided? Do these regions have any characters in common? How many **segments** or **somites** in each region? Is the number constant? Compare several specimens to determine. How do you distinguish one somite from another? Does the individual segment bear any markings? Where is the most muscular part of the body, as determined by the firmness of the body-wall? How many segments in this portion? How do they compare in size with those of other regions? In color? What is the number, counting from the front, of the largest segment of the body? Of the smallest? Do you find any segment which is not a complete ring? Has the body any protective covering or **exoskeleton**? Are there any jointed appendages, *e. g.*, legs, on the body? Are there any respiratory organs, *e. g.*, gills, visible on the surface? Any organs of hearing or of sight?

On your outline drawing indicate the various regions, placing the exact number of segments in the first two regions.

*d.* The **girdle, clitellum, or cingulum**.—Does it show equally well on all of several specimens? How is it distinguished by shape, size, color, etc., from the other regions? Does it completely encircle the body? How many segments in it? Is the number constant? Counting from the anterior end, with which segment does the girdle begin? With which does it end?

*e.* The **cuticle**.—Soak an alcoholic specimen in water for a few minutes, then with the point of a needle or with fine-pointed forceps strip some of the cuticle off the body. What is its color? Its texture? Is it easily torn? Is it flexible or rigid? Opaque or transparent? Does it cover the entire body? Does it end at the mouth and anus? In its structure is the cuticle well-adapted to the earthworm's mode of life? Examine some from the ventral surface in a drop of water and note the **cuticular sacs** in which the setæ are partly embedded. How are these sacs arranged? What is their shape? Examine the cuticle under a high power and note the **striæ** which mark its surface. In which direction do they run? To what is the color of the cuticle as seen by the unaided eye due?

Make a drawing of a portion of the cuticle as seen under a high power.

*f.* The **bristles or setæ**.—Draw the worm backward through the fingers and note the presence of the rough points. Examine them with a hand-lens. On what part of the body are they found? How are they arranged? How many in each segment?

Do you find them in *all* segments? In which direction do they point? Do *all* point in this direction? How many setæ in the girdle?

Remove a seta from an anterior segment, mount it in a drop of water on a slide, and examine under a low power. What is the shape of a single seta? How do its ends differ? Is the seta attached to the cuticle? Examine under a high power and study the structure.

Draw a seta magnified.

*g. The apertures.*

1. The **mouth**.—What is its position? What is the direction of the opening? Shape? Examine the **prostomium** or **proboscis**. What is its position? Shape? How formed? Examine the **peristomium** with a lens. What is its structure?
2. The **anus**.—Position? Shape? Compare with the mouth.
3. The **dorsal pores**.—Strip the cuticle off the dorsal surface and look for these pores in the median line in the grooves between the segments. They may frequently be detected by gently squeezing the worm between the fingers, which causes some of the fluid of the body-cavity to ooze out of these openings. How many pores does each segment have? Are all of the segments provided with them? Where is the first pore? The last?

Indicate the pores on your outline drawing.

4. The **sexual apertures**.

- (a) *The opening of the vas deferens or duct of the testis*.—With a lens look for a pair of openings,

one on each side, external to the ventral setæ in the fifteenth segment. What is the shape of the opening? Direction?

(b) *The opening of the oviduct or duct of the ovary*, situated similarly to (a), but in the fourteenth segment. Compare with (a) in all respects.

(c) *The openings of the spermathecae or seminal receptacles*, two pairs of openings situated in a line with the outer row of bristles and in the grooves between segments nine and ten, and ten and eleven, one pair in each groove. Compare with (a) and (b).

Make an outline drawing of the body as before, but of the ventral side, indicate the number of segments back to the girdle, and the openings of the sexual organs.

(d) *The capsulogenous glands*.—The swollen ventral surface of segments nine to eleven or sometimes eight to twelve is due to the presence of these glands.

*Internal Anatomy*.—Put the largest available preserved specimen into a dissecting-pan with enough fifty per cent. alcohol to cover the worm. Pin the body out straight, dorsal side uppermost, thrusting the pins through the first and one of the last segments. With a pair of sharp, fine-pointed scissors cut through the body-wall along the median dorsal line from about the seventieth to the first segment. Carefully cut the membranous partitions or **septa** at the points where they join the body-wall, and pin back the flaps of the latter. Notice that the body-wall forms a tube in whose cavity, the **body-cavity** or **peri-visceral cavity**, lies another tube, the **alimentary canal**; also that



the septa divide the body-cavity into smaller cavities. It is well to have also a second, well-hardened specimen, whose body has been divided accurately along the middle line, thus making a longitudinal section through the entire alimentary canal to show the internal arrangement of its parts.

- a. The **body-wall**. — Of what is it composed? How many layers are distinguishable? What is its color? Are all of the layers of the same color? What relation has the color of the body-wall to that of the surface of the body? What variations in the thickness of the wall? Does it contain any bony or shell-like bodies?

With a sharp knife cut a well-hardened specimen in two at the most muscular portion of the body. Then cut a thin transverse section and lay it on a slide in a drop of fifty per cent. alcohol or glycerine, and examine with a low power. Note the layers of the body-wall, especially the circular and longitudinal muscles, the latter projecting like a fringe into the body-cavity.

- b. The **body-cavity**. — How is it formed? What organs does it contain? Is it a continuous cavity? Has it any communication with the exterior? Kill a worm by drowning or by exposure to chloroform vapor under a tumbler, cut through the body-wall of some of the posterior segments, and with a pipette or a glass rod collect some of the fluid (**peri-visceral fluid**) found in the body-cavity. What is the color of the fluid? Is it very abundant? Put a drop

of it on a slide, put on the cover-glass, and examine with a low, then with a high power. Note that the fluid consists of a watery part, the **serum**, in which float numerous amoeboid cells, the **corpuscles**, as well as various *Protozoa*, small, thread-like worms, fragments of tissue, etc. The corpuscles may be stained with magenta or with acetic acid carmine if desired. Does the perivisceral fluid coagulate when exposed to the air? Draw several of the cells and other bodies found.

- c. The **mesenteric septa**.—Position? Shape? What is their relation to the constrictions between the somites in different regions? What is their relation to the alimentary canal? Why are they called “mesenteric”? What is their structure?

*A. The Digestive System.*

What is its position in the body? General shape? How do its parts lie with reference to one another? Make out the following parts in order.

The first two or three segments are occupied by

- a. The **buccal cavity**. — How formed? Shape? How are its walls connected to the body-wall? Does it contain any teeth? How is it separated from the pharynx?
- b. The **pharynx**. — Shape? Size? Through what segments does it extend? What is the nature of its wall? How is it held in place?
- c. The **oesophagus** or **gullet**.—What is its shape? Structure? In what segments does it lie? Is its calibre the same throughout its entire length? In segments eleven and twelve look for

d. The **oesophageal** or **calciferous glands**. —

How many? How arranged? To what are they connected? What is their shape? Size? Color? Structure? Examine under a low power a drop of fluid from one of these glands. What does it contain? Put one of the glands into a watch-glass or test-tube containing one part of muriatic acid to four parts of water. What result? Explain.

e. The **crop**.—Position? Shape? What is the nature of its wall? What reasons can you give for this? What is the color of the crop? What is the cause of this color? What does the crop contain (use microscope)?

Make drawings of some of the contents found.

f. The **gizzard**. — In what segments does it lie? Compare in all respects with e.

g. The **intestine**.—What is its course in the body? In which segment does it begin? Structure and color? Do the septa divide it? Note the "**liver**" (functionally, a **pancreas**) on top of the intestine. What is its shape? Color? Structure? How far does it extend? Open the intestine lengthwise a little to one side of the median line, wash away the contents carefully with a pipette, and note the folded membrane or "**typhlosole**" hanging down from the roof. Does the "typhlosole" extend throughout the entire intestine? What is its shape? Structure? Compare microscopically the contents of the posterior end of the intestine with the contents of the crop, noticing especially the amount of vegetable material, such

as fibres, groups of cells, starch-grains, etc., present in each.

Make two sketches of the entire digestive system lying in an outlined body, the first showing the digestive organs as seen from above, the second as seen in longitudinal section.

*B. The Circulatory System.*

The student will need to prepare another specimen for the examination of these organs, as some of the most important are destroyed in the examination of the alimentary canal. For this purpose kill a living worm by immersion in alcohol and examine at once. Only the more prominent vessels are given below.

- a. The **dorsal or supra-intestinal blood-vessel.**  
— Where does it begin? How far back can it be traced? What is its relation to the septa?
- b. The **circum-oesophageal vessels** or “**hearts.**”  
— Position? Number? Shape? Color?  
Lying below the alimentary canal, seen by pushing the latter to one side, find
- c. The **supra-neural or sub-intestinal vessel.**—  
Position? Relation to the “hearts”?
- d. The **lateral oesophageal vessel.** — Compare in size with the dorsal blood-vessel. Note the branches given off to the pharynx and the oesophageal glands.

Make a diagram of the portion of the circulatory system studied, showing its relation to the digestive system.

- e. The **blood.** — Open the body-cavity of a recently drowned worm and with two pairs of fine-pointed

forceps pull out one of the "hearts" in such a way as to prevent the blood escaping from the part included between the two forceps. Place this piece of the blood-vessel on a slide, put on a cover-glass, and examine immediately under a high power. What is the color of the blood? Note the **serum** and the **corpuscles**. Are the latter colored? To what is the color of the blood due? Compare with the frog's blood in this respect. What differences between the corpuscles found in the blood and those found in the perivisceral fluid? Put a drop on the slide, but do not cover. Does the blood coagulate?

Draw some of the blood corpuscles.

Remove the alimentary canal from the pharynx to the posterior end.

### *C. The Nervous System.*

Lying in front of the pharynx find

- a. The **supra-oesophageal ganglia** or "**brain**."  
— What is their position? Shape? Are they connected? What is their color?
- b. The **circum - oesophageal commissures** or "**throat-collar**." — Position? How are they formed? What do they connect?
- c. The **ventral nerve - cord**. — Position? How formed? How far does it extend? What is its relation to the intestine? Relation to the "throat-collar"?

On the ventral nerve-cord look for

- d. The **ventral ganglia**. — What is their position? Shape? How many in each somite? Look for small nerves running from each ganglion to the body-wall.

Make a diagram of the part of the nervous system studied isolated from the body.

*D. The Excretory System.*

Examine in a drop of water under a low power a septum which has been removed from near the œsophagus, and find

- a.* The **segmental organs** or **nephridia**.—Position? Number in each segment? Structure? Why called “segmental”? Do they open into the body-cavity? To the exterior? Is the internal opening in the same segment as the body of the **nephridial tubule**? On a septum removed from a living earthworm and examined in a drop of water look for the **nephrostome** or **nephridial funnel** at the inner extremity of the tubule. Note the large size of the cells forming the funnel; also the **cilia** with which they are covered. Do the cilia move? If so, of what use is their movement?

*E. The Reproductive System.*

On septa nine-ten, ten-eleven, and eleven-twelve find

- a.* The **seminal vesicles**.—What is their position? Shape? Are all of the same size? Do they vary much in size in different specimens taken at the same time of the year? In specimens taken at different times of the year? What is their color? Carefully remove the œsophagus and notice that the lateral vesicles communicate with a median vesicle in segment ten and with another in segment eleven.

There will be seen projecting into the front of each median seminal vesicle, after removing the roof of the same,

- b. The **testes**.—Number? Shape? Note the **seminal funnel** opposite each testis and prolonged into a tube (**vas efferens**), these two tubes uniting in a single tube (**vas deferens**) which opens outwardly in segment fifteen.

Attached to front wall of segment thirteen are

- c. The **ovaries**.—Position? Number? Size? Color? Compare with the seminal vesicles and the testes in all respects. Attached to the posterior wall of the same segment look for two funnels whose tubes, the **oviducts**, pierce the wall and open exteriorly in segment fourteen. Close to the point where the funnel perforates the wall look for the **receptacula ovarum**.

Look for small globular bodies in hinder portion of segments nine and ten, external to the seminal vesicles,

- d. The **spermathecae**.—Compare them with the seminal vesicles, the testes, and the ovaries. Note their openings to the exterior in the grooves between segments nine-ten and ten-eleven, close to the inner side of the outer double row of setæ.

Make a drawing of the reproductive organs, showing them in their proper segments. If facilities are at hand for doing such work, sections of embedded specimens should be made and the histology of the worm studied. This is especially instructive. For this purpose worms may be killed in strong alcohol (eighty per cent. to ninety per cent.), being left in it for twelve to eighteen hours, then cut into pieces about one inch long and some of the pieces placed in absolute alcohol for about a day. The specimens may then be embedded in celloidin or paraffin and sectioned.

## PHYSIOLOGY

While studying the live worm be careful to moisten its body from time to time with water.

- a. *Movements*.—Lay a live earthworm on a smooth surface, as on a planed board or a piece of glass. Note the manner in which the worm moves. What are its various motions? Can it move forward? Backward? From side to side?

Lay the worm on a rough surface, *e. g.*, an unplaned board or a piece of fine sand-paper. Compare the movements with those made on a smooth surface. Is progression any easier than before? Explain. Incline the board at an angle. Can the worm climb up the board? If so, at how steep an incline? Hold the worm somewhat closely in the hand and study carefully the manner in which the animal makes its escape. In what way do the setæ aid it in its efforts? Put a worm in water. Can it swim? Does it endeavor to get out of the water? Why do we find so many worms on the ground after a rain? Place the worm on the surface of the closely, but not tightly, packed earth in a flower-pot. Does the animal move any more readily than on the rough board? Explain. Note its attempts to burrow into the earth. What part of the body does it use most? In what manner is this part employed? Is this part especially adapted to the purpose in its shape, structure, etc.? How is the rest of the body employed during the act of burrowing? How long does it take to make the burrow large enough to contain the entire



**Lobster** (*Homarus Sp.*) or **Crayfish** (*Astacus Sp.*)

*Material.*—On account of their large size it is better to use lobsters than crayfishes. Live lobsters may be purchased at the markets of all cities near the seaboard, and are often to be obtained inland. They may be sent by express if packed in damp sea-weed, and will endure a journey of forty-eight hours without difficulty. On arrival the packing material should be sprinkled liberally with sea-water (artificial, if necessary), or the lobsters may be transferred bodily to vessels of such water. Should any of the specimens be found to be dead at the end of their journey, they should either be examined at once or, better, placed in fifty per cent. alcohol for a day, being careful to puncture the shell in places to permit of the entrance of the alcohol to the parts beneath, then into seventy-five per cent. and ninety per cent., each for the same length of time. They may be preserved indefinitely in the latter grade of alcohol. It is possible to use boiled lobsters, but the results obtained are seldom satisfactory. Crayfishes may be found under stones, etc., in sandy and rocky rivers and creeks throughout the central portion of the country. Except in winter, they may be caught as wanted, or, if to be used during the winter, they may be kept in aquaria of running water, supplied with plenty of aquatic plants, or in a sink into which no refuse is thrown, and be fed from time to time with small scraps of fresh meat or

bread. Injected specimens should be used for studying the circulatory system. They should be injected before being put into alcohol. Drill a hole through the carapace over the heart, puncture the latter to let out the blood, insert the canula of the syringe, and, with a firm, steady pressure, inject the starch or gum-arabic injection mass.

Other materials needed are bone forceps, coarse and fine forceps, large and small scissors, scalpels, hand-lens, compound microscope, bristles, dissecting-needles, acetic acid carmine, dilute hydrochloric acid, normal salt solution, and small piece of raw meat.

*Method of Examination.*—Live specimens should be studied in aquaria of sea-water or fresh water as required. Individual specimens may be examined in glass, earthen, or tin dishes of water. Alcoholic specimens may be laid on the dissecting-trays or in dishes of fifty per cent. alcohol. In the former case it may be well to mix about one-fourth part of glycerine with the alcohol in which the animal is preserved in order to prevent too rapid drying when exposed to the air; the glycerine will keep the tissues and organs moist and flexible.

#### MORPHOLOGY

##### *External Characters:*

- A. *The entire animal.*—What is its shape? Is it bilaterally symmetrical? Does it possess well-marked anterior and posterior ends? What is its general color? Compare several specimens to see if you can find any decided variations of shape and color. After having determined the sex of the specimen by studying the appendages (which see),

return again to this point and see if you can discover any constant characters of shape, size, etc., by which you can tell the sex. What is the average size of your specimens? Notice the hardness of the exoskeleton or "shell." Do you find all parts of the body covered by it? Do you find the exoskeleton especially modified in places? Where? To what extent? Notice that the animal consists of the **body proper** and the **appendages**. Note the position of the various parts of the body and of the appendages when the animal is at rest.

*B. The body proper.*—Note that this consists of an anterior (**cephalothorax**) and a posterior (**abdomen**) region. What proportion of the body does each occupy? As regards general structure, how do you distinguish them? Are both composed of joints or **segments**? Do both bear appendages?

Examine first,

I. **The abdomen.**—Of how many joints—*i. e.*, segments or **metameres**—does it consist? Is this number constant? How are the segments connected? Note the ball-and-socket joint at the side. On which segment is the ball? On which the socket? Are all the segments connected in this way? Are the abdominal segments all alike? Do all bear appendages? How many appendages on each segment? Can you trace any similarity of structure among the appendages? With a sharp scalpel carefully cut away from its fellows the third abdominal segment, which may be

taken as typical, with its appendages, remove the contents of the "shell," and note

a. *The segment proper*.—What is its shape as seen from the end? From the side? How is the cuticular covering or exoskeleton modified in different places? Make out the following regions on the segment:

1. The **tergum**, or dorsal portion of segment.—What is its shape? Structure? Color? What differences between its anterior and posterior edges?
2. The **sternum**, or ventral portion of segment between the appendages.—Shape? Size as compared with the tergum? Structure? Color?
3. The **epimeron**, or latero-ventral portion of segment external to appendage.—Shape? Of what is it composed? Color?
4. The **pleuron**, or downgrowth of the side of the segment.—Shape? Size? Structure? Color? Differences between anterior and posterior margins? What relation does the anterior margin bear to the preceding segment?

Near the points of union of the tergum with the pleura find

5. The **articular facets**.—What is their shape? Of what are they formed? How many are there on one segment? What relation do they bear to the preceding segment?

b. *The appendages* or "**swimmerets**."—How many on the segment? What is their position? Color? How are they attached to the segment? Make out the following parts on each appendage:

1. The **protopodite** (basal portion).—Of how many joints does it consist? How are they connected? Of what is the protopodite composed? Note
2. The **exopodite**, attached to the outer margin of 1, and
3. The **endopodite**, attached to the inner margin of 1.—What is their shape? Structure? Color? Do they show any indications of segmentation? How are they joined to 1?

Compare the first, second, fourth, and fifth abdominal segments with the third in all respects. Do you find any noticeable variations in shape, size, general structure, appendages, etc.? Do you find that all of the appendages are constructed upon the same general plan—*i. e.*, a basal portion bearing two branches? In other words, are the appendages *homologous*?

Draw the third abdominal segment and its appendages as seen from the end. Draw one of the appendages, showing its parts.

Examine next,

- II. The **cephalothorax**.—What is its shape as seen from above? From the side? Note the covering or **carapace**. Does it show any traces of segmentation? Does it entirely cover the cephalothorax? At what points is it attached to the body? What is the structure of its posterior and lateral margins? Examine the **cervical groove** running across the carapace. What course does it follow? Note that it divides the carapace into an anterior or **cephalic** and a posterior or **thoracic** region. Note also the two **branchio-cardiac** grooves running backward from the cervical

groove. That part of the carapace lying between them covers the **heart**. Examine the frontal spine or **rostrum** projecting from the carapace. What is its shape? Size? Structure? Color? How fastened to the carapace? Is it movable? Is it an appendage? Compare the rostra of several specimens. Turn the animal over on its back. What traces of segments do you find on the ventral side of the cephalothorax? Are the segments movable? Note the shape of the sterna of these segments. Examine the margin of the carapace. Does the shape of the margin bear any relation to the number of legs? With a pair of forceps or the handle of a scalpel raise up this margin, and note that a part of it, the **branchiostegite**, extending downward from the branchio-cardiac groove, is free from the body. Is this portion comparable to a single pleuron or to several fused together? Why? With a pair of strong scissors cut the branchiostegite away on one side, and note the structure of the skin lining the inner surface of the branchiostegite. What outgrowths does the skin bear? Note all the differences between the outer and inner surface of the branchiostegite. Note the cavity, the **gill-cavity** or **branchial chamber**, covered by the branchiostegite. With a pair of strong scissors make a transverse section of the cephalothorax just behind the large claws. Carefully pick away the soft parts, or clean thoroughly by boiling for five to ten minutes in caustic potash, and compare with the third abdominal segment. What differences in the shape of the tergum? Note on it the two small prominences or **endotergites** near the middle. In what way is the sternum

modified? Note the ingrowth or **endosternite**, internal to the bases of the appendage. Do you find on the abdominal segments anything corresponding to the endotergites and endosternites? Do you find any soft parts — *e. g.*, muscles—attached to the latter? Compare the epimeron with that of the abdominal segment. What difference in size? In direction? Note that it forms the inner wall of the branchial chamber, and bears an ingrowth or **endopleurite**. What is attached to the latter? If you regard the outer wall of the branchial chamber (*branchiostegite*) as composed of united pleura, do you likewise regard the inner wall as consisting of fused epimera? Why? Counting only the sterna, how many segments can you find in the cephalothorax? Do you find a pair of appendages to correspond to each sternum? Are the sterna movable upon one another?

Draw the section of the thorax as seen from the end, also from the under side.

The last section of the abdomen is called

III. The **telson**.—What is its shape? Color? What differences between the tergal and sternal surfaces? Does it bear any appendages? Note the structure of its margin. Compare it in shape, size, and structure with the other sections of the abdomen. Is the telson a segment? Note the opening (**anus**) on its under side. Try to find a hardened portion, the **peri-anal plate**, on each side.

*C. The appendages.* — By cutting through the articular

(*arthrodial*) membrane with a small, sharp scalpel, remove each of the appendages from one side of the body, beginning at the posterior end. Be sure to get the entire appendage. Examine the more delicate parts in water.

- a. The **abdominal appendages**. — Some of these have already been examined. Review them and study the others, noting in each case the structure, shape, size, etc., as well as any variations from the normal. Note that the stalk (*protopodite*) of each consists of two segments, the basal or **coxopodite** and the distal or **basipodite**; the latter forming the base, to which the other segments, the exopodite and endopodite, are articulated. Notice in every case the variations in the structure and shape of each segment, especially of the first, second, and sixth appendages. Lay each of the appendages, posterior face upward, upon a paper and draw them natural size. The appendages of the sixth segment form, with the telson, the **tail-fn**.

- b. The **thoracic appendages**.—Can you show that of the appendages on this portion of the body two groups may be made—a **posterior group**, consisting of five pairs of large appendages, and an **anterior group** of smaller appendages near the mouth? Examine the last thoracic appendage. In which direction does it extend? Does it bear an exopodite? Make out the following segments: (1) the protopodite, consisting of coxopodite and basipodite; (2) the endopodite, consisting of **ischiopodite**, **meropodite**, **carpopodite**, **propodite**, and **dactylopodite**. Note the relative



shape and size of each of these segments, and examine particularly the manner in which they are connected and the direction of motion at each joint. Can you find an opening, the **male genital aperture**, in one of the segments? Into which of the segments does it open?

Draw this appendage.

Remove the next appendage, being careful not to remove the gill attached to the articular membrane, and compare with this. Do you find that it has all of the segments named above? Does it extend in the same direction as the other? Notice that it also bears two parts not found on the other, a **gill** and a plate-like expansion, the **epipodite**. Where and how are these parts attached? Notice in female specimen of the lobster the opening of the **receptive apparatus** near the base of the coxopodite. Draw. Remove the second from the last appendage and compare with the other two. Which does it more closely resemble? Does it bear a gill and an epipodite? Note how the dactylopodite is attached to the propodite, forming with the latter a pair of pincers or a **chela**. Look for an opening, the **female genital pore**, in the coxopodite of this appendage. Draw. Compare the next appendage with the preceding, noting all the resemblances and differences. Draw.

Remove the **great chela** or **pincers** and compare with the other appendages, noting especially the great size of the segments. Are the two chelæ shaped alike? If not, do you find on examining a number of specimens that the great chela of the right side has certain constant characters and that of the left also? Are the two chelæ of the same size? Do you find any extreme difference in the size of the two chelæ on the same animal? If so, how do you explain? Do you

find differences in the teeth on the inside of the jaws of the forceps? Are these teeth fastened into sockets? Note the distribution of hairs along the margin and over the surface. Is it different from the other appendages examined? Compare several specimens to see whether or not the resemblances and differences are constant. Draw.

Can you give any reasons for considering as homologous the paired openings found on the thoracic appendages? Review the five appendages on the other side of the body and note their relative size, the various directions in which they extend, the motions allowed by their joints, etc.

Remove the next appendage in front, the **third maxillipede** or "**foot-jaw**." Can you find all the segments represented in the great chela? In the last thoracic appendage? Does it have an exopodite? A gill? An epipodite? Note the bunch of bristles, the **coxopoditic setae**. Are they on the coxopodite or epipodite? Note the teeth on this appendage. How many segments bear them? What is their arrangement on each segment? Study the distribution of the hairs. Draw. Why is it called a "foot-jaw"?

Remove the next two appendages, the **second** and the **first maxillipedes**, and compare with the preceding. Do you find any segments lacking? Which are especially modified and how? Draw each.

Review all of the thoracic appendages and notice again that they naturally fall into two groups. Give what you consider to be the most characteristic structural features of each group. On what grounds do you divide them?

c. The **head appendages**.—Remove the most poste-

body does it lie? What separates the branchial cavity from the body-cavity? At which point does it communicate with the exterior? Look for the **cervical canal**, in which lies the scaphognathite. Does the branchial cavity contain any organs besides gills? Notice that the gills may be divided into three groups: (1) **podobranchiæ**, those attached to the exopodites of the appendages; (2) **arthrobranchiæ**, attached to the articular membranes; and (3) **pleurobranchiæ**, attached to the inner wall of the branchial chamber.

Review all of the appendages and note those on which you found gills. How many arthrobranchiæ do you find? Do you ever find two on the same membrane? If so, on which? What position have the arthrobranchiæ with reference to those attached to the appendages? How many pleurobranchiæ are there? What is their position? In which group are the gills largest? Do any of them branch? Do all have the same general shape? Are the gills *within* the body? Remove in succession one of the largest gills of each group, place it in a dish of water, and examine its structure. Is the gill stiff or flexible? Do all have the same structure? If not, which are the most complicated? Notice the finest divisions, the **branchial filaments**, of the gill. In the crayfish, note that some of the pleurobranchiæ are rudimentary. Can you give reasons for calling them "rudimentary"?

Draw a gill of each kind.

*E.* The **exoskeleton** or **cuticle**.—What is its nature? Color? Thickness? Is it the same on the ap-

pendages as on the body? How is it modified at the joints? Does it bear any outgrowths? If so, where are they and what is their structure? Put a piece of the exoskeleton into boiling water for a few minutes. What changes take place in it? Put a piece into dilute hydrochloric acid, consisting of one part acid to four parts water. What happens? Explain.

*F. The organs of special sense.*

- a. The **tactile organs**—*i. e.*, the antennæ, the antennules, and the palpi on the oral appendages—have already been studied.
- b. The **eye**.—Remove one of the eye stalks, note again its gross structure, then examine with a lens. Notice its transparent outer covering, the **cornea**. What is its shape? How is the surface marked? Each area or **facet** corresponds to one of the elements of which the compound eye is composed. What is their shape? Arrangement? Estimate the number of facets. Make a longitudinal section of the eye and its stalk, cutting the hardened portion with scissors, the rest with a scalpel. Examine with a strong lens or the low power. Note the edge of the cornea; the ring of opaque exoskeleton to which it is connected by a membrane; the striated mass marked by radiating lines, indicating the **striated spindles** and the **crystalline cones**; the dark-colored central mass; the **optic ganglion**, from which runs through the axis of the stalk; the **optic nerve**, and the **muscles** lying on each side of the optic nerve. Examine as closely as

possible the relative position of these various parts, and make a drawing of the eye.

- c. The **ear**.—Among the bunches of setæ on the basal joint of an antennule find, by using a fine bristle, the opening into the auditory organ. Leave the bristle inserted and, using it as a guide, cut away the under side of the joint with a scalpel. Close examination will reveal a small transparent sac, the **auditory sac**, lying among the muscles within the joint. Dissect this out. How large is it? Examine under the low power in a drop of water or glycerine. What is the shape of the sac? Color? Cut it open. Do you find sand-like particles or **otoliths** inside? Notice also the setæ or **auditory hairs**. What is their position? Shape? Relation to the otoliths?

Draw the sac, otoliths, and setæ.

- d. The **olfactory setæ**.—Examine with the lens the under surface of each joint of the endopodite of the antennule for setæ. Are these at all different from those found at the edges of the segments?

Draw.

*Internal Anatomy*.—The various systems are given in the order in which they are most conveniently examined. Take a second specimen, and with a pair of strong shears, being careful not to injure the organs lying beneath, cut through the exoskeleton along each side of the body from the telson to the rostrum. Begin at the anterior end of the abdomen and, working back, carefully remove the upper half of the shell by cutting the muscles with a sharp scalpel at their points of attachment, noting par-

ticularly the position of these points. Use an injected specimen if obtainable.

Lying beneath the shell look for

- A. The **epidermis** or "**hypodermis**."—Do you find it everywhere inside the skeleton? What is its color? Is it more closely attached to the segments of the shell at some points than at others? If so, where are these points?  
Remove the epidermis.

*B.—The Muscular System.*

Lying one on each side of the median dorsal line find

- a. The **extensor abdominis muscles**.—Trace the two strands forward to the thorax. What is their color? Shape? Where do they arise? To what are they attached posteriorly? Notice that each is made up of smaller strands, and that these strands are attached to the terga of the segments. Are the two strands connected in any way? Are the muscle fibres attached to the epidermis or to the exoskeleton? When these muscles contract, what motion do they produce?  
Remove the extensor abdominis muscles.

Arising on the side of the carapace, directly above the origin of the extensor abdominis, are

- b. The **levator abdominis muscles**.—In what direction do these muscles run? To what are their posterior ends fastened? What relation as regards position do these muscles bear to *a*? How do they compare with *a* in shape and size? What motion do they produce?

- c. The **flexor abdominis muscle**.—The greater

part of the abdomen will now be seen to be filled with a mass of muscle, through the middle of which runs a deep groove. In the groove are the superior abdominal artery with its side branches and the intestine. With scissors divide the branches of the blood-vessel as far out at the side as possible, cut the intestine across just in front of its dilated end, and lay both blood-vessel and intestine over at one side. Turn forward the glands and other organs covering the anterior end of the muscle. Cut vertically through the mass of muscle at the line of junction of the fifth and sixth segments, and carefully work it out of the shell, taking precautions not seriously to injure the nerves lying below the muscle.

How does it compare in size with *a* and *b*? Of how many smaller masses is the muscle composed? In what direction do its fibres run? To what part of the segments are they attached? Do the fibres extend into the pleural region of each segment? To what is the anterior end of the muscle attached? For what purpose is the deep median groove? What motion does the muscle produce? How do you account for its size?

- d.* The adductor muscle of the mandible.—Lay the carapace back on the body, note the position of the cervical groove, and look directly in front of it on the inside for the origin of a fan-shaped muscle, which tapers to a tendon and runs obliquely downward to the mandible. Note the direction of the muscle. Compare the length of the muscular portion with that of the tendinous

portion. Does the tendinous portion branch? If so, to what is the branch attached? To what part of the mandible is it attached? Pull on the muscle and notice the movement of the mandible.

*e.* The **antennary and other muscles**.—Examine the basal joints of the antennæ, antennules, and ophthalmites, and note the small muscle strands running from the joints to adjacent portions of the exoskeleton. With fine-pointed scissors split these organs open lengthwise. Do the muscles extend *into* these appendages?

*f.* The **muscles of the great chela**.—With a scalpel cut through the articular membrane at the base of this appendage and remove it entire. Notice that the muscles of the basal joints are attached to ingrowths of the sternum, the **endosternites**. With a pair of bone forceps cut around the edges of the propodite and the dactylopropodite and remove the shell. Do the muscles entirely fill these segments? With a scalpel make a vertical cut, about half the thickness of the muscle, running from the angle between these two segments to the outer corner of the base of the propodite, and remove the inner half of the muscle which is thus divided. Notice the **central tendon**. How do the muscle fibres in the propodite run with relation to this tendon? Remove the remainder of the muscle mass, thus exposing the entire upper surface of the tendon. What is its shape? Size? With a pair of forceps grasp the posterior border of the tendon and pull upon it. To what is it connected at its outer end? What motion does it produce? Can



you explain its large size and the connection to it of so many muscle fibres? Why should this be called the **adductor** muscle? On the inner side of the base of the propodite find another tendon to which are attached muscle strands. Compare this tendon and muscle in all respects with the first, and give reasons for the differences found. Why is this muscle called the **abductor**? Notice that each tendon is an ingrowth of the exoskeleton at the base of the dactylopodite.

Peel what is left of the muscle mass out of the two segments, leaving the epidermis attached to the exoskeleton, and note the honeycomb appearance of the epidermis, especially near the base of the propodite. What causes this appearance?

- g. The **microscopic structure of muscle**.—From a recently killed specimen take a small piece of muscle, lay it on a slide in a drop of normal salt solution, tease the muscle carefully with fine-pointed needles, and examine under the low, then the high power. What is the structure of a muscle strand? Of one of the constituent fibres? Are the fibres striated? Draw.

Stain in dilute acetic acid carmine and note the results.

### *C.—The Circulatory System.*

Lying at the anterior end of the superior abdominal artery look for

- a. The **pericardial sinus**.—What is the shape of the sinus? With scissors divide it along the median dorsal line, and carefully fold back the flaps thus made. What is the nature of its wall? Look

for the openings where the blood-vessels coming from the gills enter the pericardial sinus. Lying within the sinus will be found

- b. The **heart**.—What is its shape? Its size as compared with the sinus? Look for the fine thread-like muscles, the **alary muscles**, extending from the corners of the heart to the wall of the sinus. How many such muscles do you find? On the upper surface look for the two **dorsal cardiac apertures**. What is their position? Shape? Size? Can you detect the lip-like **valves** guarding the openings? Remove the roof with scissors. What is the structure of the wall of the heart? Is the cavity single, or is it divided into chambers, as **auricles** and **ventricles**? On the floor of the heart look for the **ventral apertures**. How many do you find? What is their position? Do they have valves? Compare in all respects with the dorsal apertures. On the sides of the heart look for **lateral apertures**. How many are there? What is their position? Compare with the other apertures. Cut away one side of the heart down to the level of an aperture, and try to see how the valves work. Do they prevent the exit or the entrance of the blood at these apertures?

Make a drawing of the heart, showing on one side the dorsal surface, on the other the internal aspect.

Running from the posterior end of the heart find

- c. The **superior abdominal artery**.—How far does it extend? Notice the branches. At what points are they given off? To what parts do they run?

How does their number compare with that of the abdominal segments? Does it send any branches to the intestine?

Originating at the anterior end of the heart find

- d. The **ophthalmic artery**.—How does it compare in size with *c*? Trace it forward over the stomach. Does the artery give off branches? If so, where do they go?

Branching out from the heart, one on each side of the ophthalmic artery, find

- e. The **antennary arteries**. — What course does each follow? What is their relation to the adductor muscle of the mandible? To the stomach? Do you find the branch (**gastric artery**) given off by each? Can you find branches running to the kidney or "green gland," antennæ, antennules, and rostrum? Do you find any branches running to the muscles around the stomach?

At the ventral side at the anterior end of the heart find

- f. The **hepatic arteries**. — How many are there? Can you trace their course? How do they compare in size and length with *e*?

Arising at the posterior end of the heart, immediately below the superior abdominal artery, find

- g. The **sternal artery**.—In what direction does it run? On which side of the intestine does it pass? Notice that it divides into two branches, one of which, the **antero-ventral artery**, runs forward (its course should not be traced until the organs

lying in the cephalothorax have been examined and removed); the other, the **inferior abdominal artery**, runs backward. What is the relation of the latter to the ventral nerve-chain?

Make an outline drawing of the body of the animal seen in vertical longitudinal section, and indicate the course of the blood-vessels examined.

*h.* The **microscopic examination of the blood**.—

Kill a specimen, make an opening through the shell into the pericardium, put on a slide a drop of the fluid which escapes, and examine. What is the color of the blood? Does it coagulate readily? What sort of corpuscles does it contain? Draw.

*D.—The Digestive System.*

Remove all of the gills, glands, etc., on one side of the thoracic region so as to give a side-view of

- a.* The **stomach** (lying immediately in front of the heart).—What is the shape of its upper surface? Notice the muscle bands running in various directions. Endeavor to make out a pair, the **anterior gastric muscles**, passing from the anterior end of the roof of the stomach to the base, the **procephalic process**, of the rostrum. What motion do these muscles produce? Another pair, the **posterior gastric muscles**, pass from the roof of the posterior end of the stomach to the carapace and end just in front of the cervical groove. Draw the upper surface, showing the attachments of these muscles. Another large sheet of muscle fibres, the **great constrictor**, surrounds the posterior end on the ventral side. Notice that some portions of the wall of the

stomach are hard and firm, while others are more flexible. What relation exists between the muscles and the hardened portions of the stomach wall? Carefully remove the stomach from the surrounding parts, cutting off the entrance, or **oesophagus**, close to the mouth, and leaving about an inch of the intestine attached. What is the position of the stomach with regard to the mouth? Lay the stomach on its side in a dish of water or fifty per cent. alcohol, and remove all of the superfluous parts. Do you find that the wall consists of two layers? To which of these are the muscles attached? In which do the hardened portions lie? Does the outer fit closely over the inner coat? What is the shape of the stomach when seen sidewise? Draw. Examine the **oesophagus** or **gullet**. How long is it? How wide? Are its walls flexible or rigid? At what point does it enter the stomach? Can it be dilated? Is its lining continuous with the outer covering of the body? Note the two regions of the stomach proper, the anterior or **cardiac region** and the posterior or **pyloric region**. How do they compare in size? If collapsed, the stomach may be distended by injecting water into the **oesophagus**. What difference in shape?

Note the lining of the stomach. Of what is it composed? Trace it to the mouth. Remove a portion of the roof and front wall of the stomach, so as to expose the interior, being careful not to cut away any of the hardened portions or **ossicles**. Examine the ossicles and try to make out the following, studying in each case the position, shape, size, and attachments:

1. The **cardiac ossicle**, extending across roof of cardiac cavity.
2. The **pyloric ossicle**, extending across roof of pyloric cavity.
3. The **lateral cardiac ossicles**, attached to ends of 1.
4. The **lateral pyloric ossicles**, attached to ends of 2.
5. The **uro-cardiac ossicle**, passing backward from 1.
6. The **prepyloric ossicle**, passing backward and downward from 2.
7. The **gastrolith**, in the anterior cardiac wall. If this is not found, see if there is not a thickening of the cuticle in this region.

Divide the stomach accurately along the middle line with a pair of scissors, and look for

8. The **postero-ventral ossicles**, in the posterior wall of the cardiac sac. Do you find any other ossicles than those mentioned?

Make a drawing of the framework formed by the ossicles.

Look also for

6. The **gastric teeth**.

1. The **median tooth** (lying between the ends of the two median ossicles).—What is its shape? Size? Color? Does its tip touch against any other part?
2. The **lateral teeth** (attached to the lateral pyloric ossicles).—Compare with 1.

Gently pull upon the cardiac and pyloric ossicles in such a way as to imitate the action of the muscles attached to these parts. What motion do the teeth make?

Examine the opening between the cardiac and pyloric portions of the stomach guarded by the **median cardio-pyloric valve**. How is it formed? What is the nature of its surface? Which way does it open? Do you find other valves, the **lateral cardio-pyloric valves**? If so, compare with the median valve. Note the opening into the intestine. Is it also guarded by valves and setæ?

- c. The **intestine**.—If necessary, distend the intestine with water injected with a pipette into the anus. What is its general direction through the body? At what point does it leave the stomach? What is its length? How is it held in place? On the dorsal surface of the intestine, near the posterior end, find a sac-shaped outgrowth, the **cœcum**. How does it compare with the intestine in diameter? Split the intestine open by a cut made lengthwise along the dorsal surface from the anus to the pyloric portion of the stomach. Is the intestine wrinkled or folded? If so, in what direction? Is the lining membrane anterior to the cœcum similar to that posterior to the same part? Is that part posterior to the cœcum continuous with the exoskeleton? Compare with the lining membrane of the stomach.

Find a lobulated mass lying on each side of the stomach,

- d. The **digestive glands** or "**liver**."—How much of the cephalothorax does it occupy? What is its color? Look for the duct by means of which the gland connects with the intestine. Where does the duct enter the intestine?

*E.—The Excretory System.*

Lying in the lower side of the head will be found

- a. The “**green glands**” or **kidneys**.—What is their position with reference to the mouth? How do they compare in size and color with the digestive glands? Is there any similarity in the general structure of the two kinds of gland?

Leading from the green glands to the base of the antennæ find

- b. The **ureters**.—What is their shape? Length? Pass a bristle into the opening in the tubercle on the base of the antenna. Does the bristle enter a ureter?

*F.—The Reproductive System.*

In the male find

- a. The **testes** (in the lobster, two tubular organs extending from the lateral angles of the stomach back into the abdomen; in the crayfish, an organ bilobed anteriorly, but with a single lobe posteriorly).—How far back do they extend? What is their color? What is their position with regard to the intestine? To the heart? What holds them in position? Are the two organs connected at any point?

Cut into one of the testes of a recently killed specimen, place a drop of the contained fluid upon a slide, and examine with a high power. Examine the shape and general appearance of the sperm-cells or **spermatozoa**. Do they move? Draw some of these cells.

Leading from the testes find two tubes,



- b. The **vasa deferentia**.—What is their shape? Size? In which direction do they run? Where do they open?

In the female find in a position and of a shape quite similar to the testes,

- c. The **ovaries**.—Compare in every respect with the testes. If the ovaries contain eggs, examine them in different portions of each gland to see if there is any difference in size. Make a longitudinal cut into the ovary, wash with a gentle stream of water, and then look for the **ovisacs**, in which the eggs are contained. Examine under a lens. Place some of the eggs on a slide, stain with magenta, and examine. Draw.

Find also

- d. The **oviducts**.—Compare these with the *vasa deferentia*.

Remove the muscles and digestive organs from the specimen, also the **endophragmal system** (a complicated set of ingrowths from the cuticle of the ventral surface), and study the principal portions of

#### G. *The Nervous System.*

At the base of the rostrum look for

- a. The **supra-oesophageal ganglia** or "**brain**."—What is their shape? Are they connected with each other? Do they give off any branches? If so, where do these branches run? Look for nerves passing from the "brain" to the eyes, antennules, and antennæ.

Behind the gullet find

- b.* The **sub-oesophageal ganglion**.—Compare with *a* in shape and size.

Is there any connection between the ganglion and the “brain”?

- c.* The **thoracic ganglia**.—How many are there? How are they situated? Are they connected with one another? How do they compare in shape and size with *a* and *b*? How many in each segment? To what parts do their branches run?

- d.* The **abdominal ganglia**.—Compare in all respects with *a*, *b*, and *c*.

Make a diagram showing the shape and relation of those parts of the nervous system mentioned above.

#### PHYSIOLOGY

The living animals should be studied in a pan of water or in an aquarium. Though living specimens are preferable, it will be possible to do much of the following work upon the dead animal.

- a.* **Movements**.—Can the animal walk? If so, in how many different directions? At how rapid a rate? What appendages are used for this purpose? Do all of these appendages move in the same manner? Do all occur on the same region of the body? Can you give any reason why they should be here? Do all seem to be adapted to this particular function? Does the animal swim? By means of what organs? Where are these located? Why are they here? How do they work? In what direction does the animal swim? Why in this direction? Does it use both ambulatory and swimming organs at the same time?

How rapidly can it swim? Has it any other modes of locomotion than walking and swimming? Is its body adapted for rapid motions? For walking and swimming long distances?

1. *Movements of the regions of the body.*—What region of the body is most flexible? In what direction does it bend? To what extent can it bend in this direction? Of what use is this motion? Can it bend in the opposite direction? Why?
2. *Movements of the appendages.*—Examine each appendage separately. What motions have the abdominal appendages? Do all have the same? Is their range of movement very great? Of what use are their movements?

Of what movements are the last five cephalothoracic appendages capable? Can you describe a circle with their tips? By what sort of joint are their segments connected? How is it possible with this kind of joint to produce the range of motion possessed by these appendages? Notice the peg-shaped outgrowth upon one of the basal joints of the chelæ. What is its use? Study the movements of the dactylopodites. For what purposes are the various forceps used? What are the motions of the maxillipedes and the maxillæ? Is there any advantage in this? Turn the animal over on its back. How does it right itself? What appendages are used?

What difference between the lobster's jaws and yours in their direction of motion?

In what different directions can the antennæ point? The antennules? In what position are

the former usually carried? In what the latter? What advantages arise from these movements? Is the scale-like appendage of the antenna mobile? Examine the eyes. How great is their range of motion? Can the lobster look backward? Down by its side? Upward? Can it see what it is eating? Can it close its eyes? Are any of the movements such as to protect the eyes?

Watch for the motions of the scaphognathite. In what manner does it move? At what rate? Of what use are its movements? Can you make out whether or not the gills move? Does the branchiostegite?

*b. Feeding.*—If the lobster is studied, put small pieces of meat, clam, bread, sea-weed, etc., in the aquarium; if the crayfish, use meat and bread. If not too large, put the animal into a glass dish, so that by holding it up you can see the motions of the mouth parts. How does the animal seize its food? How convey it to the mouth? While eating one piece will it seize another? Attempt to remove a piece which the animal is holding. What does it do? What is the action of the chelæ? Of the maxillipedes? Of the maxillæ? Is the food thoroughly chewed before being swallowed? Does the animal “wash down” its food with a swallow of water?

Examine again the stomach of a dead specimen in water and try to discover the action of the various gastric ossicles, and how their action supplements the work of the jaws. After food has once been swallowed, what prevents it from

dropping out of the mouth again when the next mouthful is swallowed? Do you find any structures whose function may be to separate the larger, undigested particles of food and prevent their entrance into the intestine?

Are the lobster and the crayfish structurally adapted to capture living animals for food? Is either adapted for catching as prey animals having quick, active movements?

If living specimens cannot be obtained, examine the contents of the stomachs of dead ones.

- c. *Breathing*.—Place the animal in a dish of water and allow it to become quiet. Then with a pipette run into the water, close to the bases of the hinder thoracic appendages, a few drops of water containing particles of indigo, carmine, or India ink. Note the direction of the current. Do the particles enter the branchial chamber? If so, at what place? Where do they come out? To discover the cause of this current, with a pair of strong scissors open the cervical canal by making two cuts: the first directly behind the cervical groove, the second about a half-inch in the lobster, or one-fourth inch in the crayfish, back of the first and parallel to it. Then remove that part of the branchiostegite included between the cuts. This operation can be done with little or no inconvenience to the animal, and permits the action of the scaphognathite to be seen. Watch the motions of the last. How does it cause a flow of water over the gills? Examine a specimen in which the legs are still attached to the thorax. Move one of the legs. Does the gill or podo-

branchia move with it? Do you think the podobranchiæ are moved when the animal walks? Can you discover that the epipodites have any function?

*d. Nervous properties.*

1. *Touch*.—With a long bristle gently touch the animal in the following places, noticing each time whether or not, by moving or otherwise, it gives evidence of feeling the touch: The dorsal surface of the carapace, the dorsal surface of the various abdominal segments, the telson, the lower edge of the branchiostegite, the hairs on the various appendages of the thorax, any of the mouth parts within reach, various points from the tip to the base of the antennæ, likewise of the antennules, the stalk and the surface of the eye, and the rostrum. Is the sense of touch distributed over the entire surface of the body? Are there any appendages which are especially sensitive to touch? If so, can you give any reasons for this? Do the hairs on various parts of the body show different degrees of sensibility to touch? Are any of them so sensitive as to warrant regarding them as "tactile hairs"? If so, where are they and why should they be so situated?
2. *Taste*.—Put a piece of bread and a piece of meat in the water near the animal. Does it show any preference for either of these? Try a piece of fresh and a piece of decaying meat. Which does the animal prefer?
3. *Smell*.—Lay a piece of meat in the bottom of a

dish containing a live lobster or crayfish which has had nothing to eat for three or four days, and, at some distance from the animal, with a pipette quietly force a current of water from the meat towards the animal. Does the latter show by its actions that it is conscious of the presence of the meat? How does the animal behave? Do you notice any peculiar motions of the antennæ or antennules? How long before it recognizes the presence of the meat? Can you decide definitely whether its actions are due to the sense of smell or that of taste?

4. *Sight*.—Find a specimen which is lying quietly near the side of the aquarium. Move your hand quickly past the animal. Is it at all disturbed? Watch several which are moving around. Do any of them show that they can see the others coming from a distance? Do the eye-stalks move in such a manner as to permit the animal to look in different directions?

*General Questions*.—Considering the appendages morphologically, in what various ways do you find segments of the typical appendages modified? Examine again all of the appendages, and write a list of the modifications of structure found on each. From this list make another, giving all of the variations found. Is any one segment more susceptible to variation than the others? If so, can you give any reasons for it?

Review all of the appendages from a physiological rather than a morphological point of view. Examine each appendage by itself, and write out a list of all the functions which you *know* each appendage to be capable of performing. Then from this list make a second,

showing the various functions which a typical appendage, though modified in different ways, may perform. Do you find here a close relationship between *structure* and *function*? Can you determine whether the *structure* of an organ determines the *function* which it can perform, or *vice versa*?

What means of protection from its enemies has the animal? What means of defence? Of offence? When the animal is moving along, does it co-ordinate the movements of its various appendages—*i. e.*, do all of the appendages work together for the accomplishment of a certain object? Turn a live specimen over on its back. In righting itself, does the animal show co-ordinated motions of the appendages?

Compare the lobster or the crayfish with the earthworm as regards the plan upon which the body is constructed, the collection of the segments or metameres into well-defined regions, the arrangement and structure of the appendages, the number, position, and structure of the sense organs, the structure of the muscular, the nervous, and the digestive systems, etc. Which is the more complicated or "higher" animal? Why?

Use the crab (*Cancer* or *Callinectes*) for comparison.



### **Locust (*Caloptenus Sp.*)**

*Material.* — Locusts, generally miscalled “grasshoppers,” may be found in greater or less abundance in fields and along roadsides, from midsummer until the early frosts come. The largest specimens available should be caught. Each student should have two or three specimens of each sex. If this type is to be studied at a time of year when living insects cannot be obtained, a sufficient number of them should be preserved in alcohol. They may be put when first caught into three to six times their bulk of sixty per cent. alcohol for a day, and then transferred to about the same quantity of eighty per cent. to ninety per cent. for keeping.

It is impracticable to try to keep these insects alive during the winter.

For the examination the student will need fine forceps, small scalpel, fine scissors, hand-lens, dissecting-needles, chloroform or ammonia, fifty per cent. alcohol, metric scale, compound microscope, dilute hydrochloric acid, and normal salt solution.

*Method of Examination.*—Preserved specimens may be studied without any further preparation. It is well, however, to remove them from the strong alcohol in which they are kept to a mixture of equal parts of fifty per cent. alcohol and glycerine for about an hour before beginning the examination. The stiffened muscles be-

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come flexible and the drying of the specimen is prevented by the glycerine.

Let the student lay the specimen before him on the table with the back uppermost and the head turned away from him, and make out as many as possible of the characters. Then the body may be studied in different positions, the appendages removed and examined, longitudinal and transverse sections made, etc.

Specimens may be killed by pouring a few drops of chloroform, ether, or ammonia on the body.

Living specimens may be placed for study in glass jars or fruit-cans, into which a few blades of fresh grass have been thrown.

#### MORPHOLOGY

- a. *The shape of the body as a whole.*—What is the general shape? Does it resemble that of the crayfish or lobster? Is the body bilaterally symmetrical? Is the insect's shape at all related to its mode of life? How many regions are there in the body? Compare with lobster and crayfish. How distinguished from one another? Are they more or less plainly marked off from one another than in the lobster or crayfish? Do all of them bear appendages? Compare with crayfish. How many pairs of appendages do you find? How many kinds of appendages are there? What character have the appendages in common? Is there an exoskeleton? If so, does it in any way resemble the lobster's? How many segments or indications of such are to be found, without dissection, in each region? Compare with lobster. How long is your specimen? Measure several specimens to

find the average size. How many colors do you find on the body? How are they distributed?

*b. The head.*—What is its shape as seen from the front? From the side? From above? How is it connected to the thorax? Is there a movable neck? How many pairs of appendages does it bear?

On the head find

1. The **compound eyes**.—How many? How situated? Are they upon eye-stalks? Compare with crayfish. What is their color? Shape? Size? Examine with a hand-lens. Estimate the number of facets in the eye. What is the shape of the facets? Examine the compound eye as an opaque object, under the low power.
2. The **ocelli** or **simple eyes**.—How many? How situated? Color? Shape? Size? Compare in all respects with the compound eyes.
3. The **antennæ**.—Number? Position? Structure? Color? Shape? Size? Compare with crayfish. How do you account for the difference in the length of the antennæ of the two animals? Examine with a hand-lens and count the joints. Examine several specimens to see if the number is constant. Are there any variations in the size of the joints? Are the antennæ true appendages? Why? Are the antennæ of the locust homologous with those of the lobster?
4. *Cephalic plates.*

Make out the following plates composing the head :

The top and front of the head are formed by

(a) The **epicranium**.—What is its shape? What

proportion of the head does it cover? What is its color? Note the central ridge with a simple eye or **ocellus** in the middle line.

Fastened to the lower edge of the epicranium is

- (b) The **clypeus**.—How does it compare in size with the latter? Does it consist of a single piece? Does it bear any ridges?
- (c) The **genæ** or "**cheeks**."—Which of the above structures do they touch at their edges? What is their shape?

Below the clypeus is

- (d) The **labrum** or "**upper lip**."—Raise it up with the point of a pin. To what is it attached? What is its shape? What range of motion has it? Draw.

##### 5. *Mouth parts.*

As was done with lobster or crayfish, remove and study also the following

- (a) The **labrum** (already examined).
- (b) The **labium** or "**under lip**."—Compare in every respect with the labrum. Does it look as though it were made of two parts which had fused together along the middle line? Try to make out the following parts: the **submentum**, composed of two protopodites fused together, the hinder curved portion forming the **gula**; the **mentum**, which is the fused distal joints of the protopodites and bears the endopodites, which together form the **ligula**; the **palpi** supported on a stump-like base or **palpiger** and representing the exopodites.
- (c) The **labial palpi**.—How many? Where and how are they attached to the labium? Are

they movable? Of how many joints does each consist? Are the palpi appendages? Draw the labium with its palps attached.

(d) The **maxillæ** or "**soft jaws**."—Number? Position? Does a maxilla in any respect resemble the corresponding part of the lobster? See if you can find the protopodite consisting of **cardo** and **stipes**, the endopodite consisting of the **lacinia** and the **galea**. What is the exopodite?

(e) The **maxillary palpi**.—Compare these in every respect with the labial palpi, and draw a maxilla with its palp.

(f) The **mandibles** or "**hard jaws**."—Compare in every respect with the maxillæ. In which direction do the mandibles open? To what are they connected? Draw.

Push the outer mouth parts aside and find

(g) The **tongue**.—Study its position, shape, size, structure, etc. Does it bear appendages?

Does the head show indications of being composed of segments? If so, of how many? In the locust is cephalization carried to a greater or less extent than in the lobster? Why? Judging from the number of pairs of appendages or traces of such, of how many segments does the head consist?

Make enlarged drawings of the head as seen from the front and from the side, showing in each case all of the structure visible.

c. *The thorax*.—What proportion of the body does it form? Of how many distinct pieces is it composed? What appendages does it bear? How are they situated?

Make out the following parts:

The first segment forming

1. The **prothorax**. — What is its shape as seen from above? From the side? How is it connected to the parts preceding and following it? How does it compare in size with the other segments of the thorax? Does it bear appendages? Notice the expansion, the **pronotum**, of its tergal portion. Try to make out the following plates composing the prothorax: on the dorsal side, beginning at the anterior end, the **præscutum**, the **scutum**, the **scutellum**, and the **postscutellum**; on the ventral side, parts corresponding to the sternum of the lobster, a soft, flexible membrane with a posterior hardened piece. Note the hairy tubercle, also at the side in the membranes connecting the prothorax and the mesothorax, the opening or **spiracle**. Examine the **legs**. Number? Color? Position? To what parts are they attached? In what direction do the joints bend? Examine a leg carefully and make out the following parts: **coxa**, **trochanter**, **femur**, **tibia**, and **tarsus** or foot. Study carefully the structure of each part. Draw one of the legs.

Draw the prothorax, showing dorsal, ventral, and side views.

Examine the second joint or

2. The **mesothorax**. — Shape? Connections? What appendages does it bear? How are they situated? Does this segment bear any spiracles? Compare in all respects with the prothorax. Find the two side plates, the **episternum** in front and the **epimeron** behind, comprising

this segment. What is the shape of the scutum? Of the scutellum? Of the sternum? Note the wings or "**wing covers**." Where and how are they attached? What is their structure? Color? Shape? Size? Compare the legs with those on the prothorax.

Draw the mesothorax, showing all the parts.

3. The **metathorax**. — Compare this segment in shape, size, structure, etc., with the preceding segments. Compare also the appendages, both the legs and the wings, with the similar appendages on the other parts of the thorax, noting especially the shape, size, texture, folding, venation, color of the wings, and the shape and arrangement of the joints of the legs. Are there any spiracles on this segment? If so, where?

Draw the metathorax, also the appendages.

- d. *The abdomen*.—How is this connected to the thorax? Shape? Size as compared with the head and the thorax? What is its structure? Count the segments on the dorsal and on the ventral side. How does the number on the upper side compare with that on the lower? Does this region bear any appendages? Examine the first segment of the abdomen. Does it have both tergum and sternum? Note the large opening, the "**ear**," with the membrane or "**drum**" stretched across it. How many "**ears**" are there? Does this segment bear a spiracle? If so, what is its position? Note on each side of the abdomen a longitudinal fold. Pull the tergal and sternal regions slightly apart. Is this fold composed of a flexible membrane?

Examine the spiracles or **stigmata**. How many do you find on each segment? What is their position? Shape? Note the variations in the last few segments of the body in the two sexes. The females can be distinguished as having the abdomen terminate in four curved prongs. Examine such a specimen. How does the number of sterna differ from the number of terga? In the female the last sternum forms the **subgenital plate**. Compare it in shape and size with the other sterna. At the posterior end of the subgenital plate is the **egg-gulde**. What is its structure? Study the structure of the **ovipositor**, which consists of the two pairs of curved appendages already mentioned, together with a third pair lying between the first two. Notice the shape, size, and structure of each pair, their direction of motion, etc. To which segment is each pair attached? Find lying above the base of the uppermost pair a tergum for which there is no corresponding sternum. At the base of each of these curved appendages is a small plate, the **podical plate**. Find the opening, the **anus**, between them. What relation does this bear to the opening of the oviduct? Just below the podical plates are two other organs, the **cerci**. To which segment are they attached? Compare these with the parts of the ovipositor. Compare the abdomen of a male with that of the female. How many segments in the entire body?

Draw the abdomen of each as seen from the side and from above.

*Internal Anatomy.*—Use, if possible, recently killed



specimens. In studying the internal structure of the locust, the two specimens are to be used in the following manner: With the first one, cut off the wings, cut the body in two lengthwise with a very sharp scalpel, then, insect in hand, study the features mentioned hereafter. After having done this, prepare the second specimen as follows: Remove the wings, fasten the insect, ventral side downward, in the dissecting-dish containing fifty per cent. alcohol, by putting pins through the last abdominal segment and each of the hind-legs, turning the latter outward. Then begin at the posterior end, and with fine, sharp scissors cut the skin along each side of the body above the spiracles, turning the skin forward as it is loosened, thus unroofing the body. Be especially careful not to injure the heart, which lies just below the skin. Then proceed to verify the facts learned from the first specimen.

a. The **integument**.—Of how many layers is it composed? Can you find both **epidermis** and **cuticle**? What differences in their thickness, texture, flexibility, color, and arrangement on the body? Does the integument bear any outgrowths corresponding to the “hairs” found on the lobster? If so, do they correspond in general position to those on the latter animal? Put a piece of the integument into dilute hydrochloric acid. Do you get the same results as you did with the lobster’s shell? Does the integument of the insect contain carbonate of lime?

b. The **heart** (lying just under the skin).—Position? What is its relation to the ridge running down the middle of the back? Shape? Structure?

Color? How far does it extend? Does it give off branches?

c. The **abdominal muscles**.—Some can be seen only after the removal of the digestive and reproductive organs. Position? Arrangement? In what direction do their fibres run? Color? Attachments? Put a small piece of muscle tissue into a drop of normal salt solution and examine under the high power. What is the microscopic structure of the insect's muscle?

d. The **wing muscles**. — Position? Arrangement? Number? Direction of fibres? To what are they attached at each end? How are those on opposite sides of the body separated from each other? Is there any such separation between the wing muscles and the abdominal muscles?

Lying beneath the abdominal muscles find

e. The **corpus adiposum**.—What part of the body does it occupy? Structure? Color? Examine some of it under a low power.

f. The **tracheæ**. — If a recently killed specimen be placed in water the tracheæ will appear as delicate, glistening white tubes. What is their relation to the spiracles? Position? Shape? Make out if possible *six* longitudinal tracheæ: two spiracular, two dorsal, two ventral. Examine some of them under a low power and note their structure. Are they open or closed? If open, what keeps them so?

With the fine forceps pick away all of the muscles in the thorax and abdomen and find

g. *The Digestive System.*

It consists of the following parts in order, proceeding from before backward:

1. The **œsophagus**.—What is its position? In which direction does it extend? What is its shape? Color? Notice the opening, the **occipital foramen**, by means of which the cavity of the head communicates with that of the thorax. Note also the large muscles running from the inner ends of the jaws up into the head. To what are they attached?
2. The **crop**.—In what part of the body does it lie? What is its relation to the œsophagus? Shape? Color? Structure? Examine its contents with the microscope. What does it contain? Look for small **salivary glands** among the muscles under the crop, and, if possible, trace the **duct** which places the glands in communication with the mouth.

Hidden by the anterior ends of the gastric cœca find

3. The **proventriculus** or "**gizzard**."—Relation to the crop? In what segments does it lie? Shape? Structure? Color?
4. The **ventriculus** or **stomach**.—Position? Shape? How is it connected to the proventriculus? Structure? Color?
5. The **gastric cœca**.—What is their relation to the proventriculus and the ventriculus? How many are there? What is their shape? Structure? Color? With what do they connect?
6. The **ilium**.—Relation to the ventriculus? How long is it? What is its shape? Color? Structure? In what direction does it run? Do you find in it anything resembling a valve?

At the junction of the stomach and ilium look for

7. The **Malpighian** or **urinary tubules**.—To what are they connected? Are they few or many in number? What is their shape? Color? How far into the anterior end of the body do they extend?

The continuation of the ilium forms

8. The **colon**.—Position? Shape? Size? Color? Structure?
9. The **rectum**.—Compare in all respects with the colon. Look for the **rectal glands** surrounding the upper end of this portion of the alimentary canal.

Make a drawing showing a vertical longitudinal section of the digestive system.

Examine female specimens for the ovaries and oviducts.

- h. The **ovary**.—Position? Shape? Size? Color? Look for **eggs**. How many are found? What part of the body do they occupy? What is their shape? Size? Color? Examine under the low power.
- i. The **oviducts**.—How many? What is their relation to the ovary? To the posterior portion of the alimentary canal? Where do they open to the exterior?

In male specimens endeavor to find

- j. The **testes** and the **vasa deferentia**.—The former lie on the intestine in the third, fourth, and fifth abdominal segments. Compare these parts with the reproductive organs of the female.
- k. The **air-sacs**.—What is their relation to the tracheæ? Look for two very large sacs in the prothorax. What is their position? Shape? Size? How

many in the rest of the thorax? How many in the abdomen? Compare them with those in the thorax.

Remove all of the muscles and digestive organs from the thorax and abdomen.

*l. The Nervous System.*

The principal parts of this system are the following:

Lying on top of the oesophagus, between the compound eyes, find

1. The **supra-oesophageal ganglion** or "**brain**."  
—What is its shape? Size? Color? Compare with the "brain" of the lobster.

Running from the "brain" to the eyes are

2. The **optic nerves**. — What is their shape? Length?

Lying below the oesophagus look for

3. The **infra-oesophageal ganglion**.—How connected to the supra-oesophageal ganglion? What is its shape? Size?

On the side of the crop are

4. The **gastric ganglia**.—What is their shape? Size as compared with the supra-oesophageal ganglion?

Lying on the floor of the thorax are

5. The **thoracic ganglia**.—How many are there? How are they connected? What is their shape? How do they compare with the "brain" in size?

Lying on the floor of the abdomen are

6. The **abdominal ganglia**. — How many are there? How does their number compare with that of the abdominal segments? How are they connected? In what segments do they

lie? How do they compare in shape and size with the thoracic ganglia?

How does the nervous system of the locust compare in structure with that of the lobster?

What resemblances and differences do you find?

Make a diagram of the nervous system as seen from above.

*m. The thigh muscles.*—With a pair of fine scissors or a sharp scalpel cut the integument along the posterior edge of the femur of one of the third pair of legs, carefully turn back the outer flap thus formed, and notice the arrangement of the muscles within this segment. Judging from the direction of the groups of fibres, how many distinct muscles does this segment contain? Are any of the groups arranged to correspond with the peculiar tile-like pattern seen on the outer surface of this segment?

#### PHYSIOLOGY

Many of the following questions may be answered as well from a study of dead as from living specimens.

*a. The body as a whole.*—Is its shape at all related to the animal's mode of life? What advantages arise from having the body made of segments? What reasons can you give for the grouping of the segments into regions? Why are the appendages jointed? What appears to be the principal function of each region, as determined from its structure and appendages? Has the insect any skeleton? If so, how does it compare structurally

with that of the lobster? How is the body protected? What modes of locomotion has the animal? What reasons can you give for the various colors found on the body? Do you find any parasites—*e. g.*, mites—on the outside of the body, or worms within? What means of protection from its enemies has the locust?

*b. The head.*—What range of motion has the head? Is it segmented? Why? Give reasons for its mode of connection to the thorax. Why are there both simple and compound eyes? Why are they situated as you find them? In what directions can the locust look? Can the insect wink? How does it close its eyes? What is the probable use of the antennæ? Why are they jointed? What reasons can you give for their position? How do you account for the structure of the labrum? Of the labium? Put a living locust under a tumbler, and feed the insect with a few blades of fresh grass. In what direction do the jaws move? Why? What is the use of each kind of jaw? When standing on a flat surface, can the locust reach down to that surface with its mouth? What is probably the use of the palpi? Do they assist in feeding? Watch closely to see if they do not pick up the food (grass), and hold it while pieces are being bitten off. Are there any nostrils?

*c. The thorax.*—What is the function of the prothorax? Of the tubercle? What reasons can you give for the structure of the dorsal portions of the mesothorax and metathorax? Of the ventral portions?

What uses have the first pair of wings, or the "wing covers"? The wings? How does their structure fit them for their use? For what purpose are the veins in the wings? How are the wings folded? Unfolded? What is the use of the first pair of legs? Second pair? Third pair? Of the spines on the tibia of the third pair? Of the projections of the femur at the side of the joint between the femur and tibia? Of the hooks and pads? What is the position of the wings and legs when the insect is at rest? When preparing to leap? When flying? When feeding on a blade of grass? Why do the joints of the various legs bend in so many different directions? Compare with the lobster. Why are the wing-muscles so large?

d. *The abdomen.*—Why is it segmented? Of what motions is it capable? Why is there a membrane between adjacent segments? Hold a living locust in the fingers and study its mode of breathing. Give reasons for the structure of a single segment. What is the use of the spiracles? Why are there so many? Can a locust be drowned by holding its head under water? How does the insect "sing"? What is the use of the air-sacs? Why are there no external ears? Why should not the ears be on the head? How does the shape of the ovipositor fit it for its use? What reasons can you give for the presence of such a complicated digestive system? Do you find that it contains much or little food? Examine some of the contents under a microscope. Of what does the food consist?



*General Questions.*—What structural relationships do you find existing between the lobster and the locust? Do you find any structural features which these two animals possess in common with the earthworm? What do you consider to be the main points in which the last differs from the other two?

Use any butterfly or moth, or a beetle, bee, or cricket for comparison.

## MOLLUSCO SHELLS

### EXAMPLE 1.—**Fresh-water Mussel** (*Unio Sp.* or *Anodonta Sp.*)

*Material.*—Mussels may be found in rivers, ponds, and lakes throughout the country. If more convenient, clams may be used. These may be had from fish-dealers and restaurants everywhere. The shells may be preserved dry or in alcohol. For their study the student will need nothing but a dish of water, some dilute hydrochloric acid, a hand-lens, and a test-tube or tumbler.

Oyster-shells will be especially interesting for comparison.

*Method of Examination.*—Remove the animal from its shell, noting carefully the points where the connection between the shell and soft parts is closest. If, as will be the case with the living animal, the shell should be difficult to open, place it in warm but not boiling water for a few minutes. Before studying the other structural features, put some of the shells, if dry, into water to soften the hinge-ligament, and some pieces into the dilute hydrochloric acid in a test-tube or tumbler.

## MORPHOLOGY

- a. *The entire shell.*—What is its shape? Where are the points of its greatest length, breadth, and thick-

ness? Of how many parts or **valves** does the shell consist? How are the valves held together? Can you distinguish a right and a left side to the shell? Dorsal and ventral sides? Anterior and posterior ends? Do you find that some of the shells of the same kind are more convex than others? Of what use is the shell? Put a small piece into weak hydrochloric acid. What is the result? Compare with the "shell" of the lobster and the integument of the insect. How is the mussel shell probably formed?

Notice that a portion of the shell is usually covered with a deposit of mud. Which portion is it? How thick is the deposit? What is the direction of the line between the clean and the mud-covered portion? Does the latter portion show more or fewer signs of wear, such as absence of "epidermis," fading of colors, smoothness of surface, etc., than the other?

*b. The single valve.*

On the *outside* study

1. The **shape**.—What is the shape of its outline? Are the two ends shaped alike? The margins? Where is the valve thickest? Where thinnest?
2. The **epiostracum** or "**epidermis**."—Does it cover the entire valve? What is its color? Are there any variations in color? Where is this covering thickest? Can the epiostracum be removed from the shell? What is its use?
3. The **hinge-ligament**.—What is its position? What reason can you give for this? What is its shape? Of what is it made? Soak it in water, if the shell be dry, until the ligament

becomes flexible. What is its use? Why are the shells of dead mussels and clams always open?

4. The **lines of growth**.—What is their position? Shape? Number? Where do they begin? Is their number the same for the two valves of the same shell? Are they everywhere at a uniform distance apart?
5. The **umbo** or "**beak**."—What is its position? How is it formed? How does it compare in age with the rest of the shell? Does it differ in color from the other regions? If so, explain.  
On the *inside* find
6. The **hinge-teeth** (not present in *Anodonta*).—What is their position? Shape? Structure? Number on each valve? Note the two kinds—the one pointed, the **cardinal teeth**; the other elongated, the **lateral teeth**. How many of each kind are there on each valve? What is their use?
7. The **lining of the valve**.—What is its color? How does it differ from the outside of the valve? Are there any variations in color? If so, to what are these due?
8. The **pallial impression**.—What is its position? How distinguished? What is its relation to the impressions of the adductor muscles?
9. The **impressions of the adductor muscles**.—How are they distinguished? What is the position of each? Color, shape, and size of each? Note also the *paths of shifting* of these muscles.  
In front of the posterior adductor impression find
10. The **scar of the posterior retractor muscle**.—Compare with preceding.

Find also opposite the upper end of the impression of the anterior adductor muscle

11. The **scar of the anterior retractor muscle**.—  
Compare with the preceding.

Opposite the lower end of the impression of the anterior adductor muscle find

12. The **scar of the protractor pedis muscle**.—  
Compare with the above.

Break a valve in two, examine the broken edge with a hand-lens, and note the structure of the valve.

Make a drawing of the outside and inside of each valve, and of a transverse section through the thickest part of one of the valves.

**EXAMPLE 2.—Pond Snail (*Lymnaeus Sp.*)**

*Material*.—This snail is abundant in ponds and slow-flowing streams. Its large size makes it especially favorable for the study of the univalve shell. For comparative study use the shells of any land or water snails that may be had. A penknife or dull scalpel and a hand-lens will be needed.

*Method of Examination*.—Before removing the body from the shell (which may be done by dropping the snail into hot water for a few minutes, then picking out the body with a pin or needle), closely observe the manner in which and the places where the body is connected to the shell. Notice also how far back in the shell the body lies when completely contracted.

With the penknife prepare longitudinal and cross sections of the shell to show the structure of the columella. When applicable use the questions given for *Unio* or *Anodonta*; also notice

- a.* The **whorls** or turns of the spiral.—How many are there? What is their arrangement? What variations in shape, size, and color do you find? Trace the **suture** or line of junction between two whorls.
- b.* The **body-whorl** or first turn of the spiral.—What is its position? Shape? Size as compared with others? How much of the entire shell does it form?
- c.* The **spire**.—What is its structure? What is the number of whorls composing it? What is the direction of the spiral?
- d.* The **apex**.—What is its structure? Compare in age with other parts of the same shell, also compare with the umbo of *Unio* or *Anodonta*.  
Cut a shell in two lengthwise and notice
- e.* The **columella** or axis around which the whorls turn.—How is it formed? Does it extend throughout the length of the shell? Is it solid or hollow?
- f.* The **mouth**.—What is its shape? Is it the largest part of the cavity of the shell? Notice the **internal lip** or side towards the columella, and the **external lip** or side away from the columella.
- g.* The **peristome** or rim of the mouth.—What is its shape? Size? Does it bear any outgrowths, as teeth, etc.?
- h.* The **muscular impression**.—Where is it? What is its shape? Color? How is it formed?

Draw the shell as seen from the front, also a longitudinal section.

### **The Soft Parts of the Fresh-water Mussel**

*Method of Examination.*—If the animal be living, open the shell as directed under the head of “Mollusc Shells.” Remove the right valve by cutting through the adductor muscles at their points of attachment to the valve. Examine the specimen in a dissecting-pan containing fifty per cent. alcohol, which should be removed as often as it becomes turbid. If freshly killed specimens be used, the alcohol will coagulate the slimy excretion on the surface of the body. This may be cleaned off with a camel’s-hair brush.

#### *External Anatomy.*

- a. The **relation between the body and the shell.**—Does the body fill the shell? Is it in contact with the shell at all points? What holds it in place? Does it have the same shape as the shell? How do you distinguish the anterior end of the body?
- b. The **pallium or mantle.**—Why is it so called? What is its shape? How many lobes or folds has it? Are they connected? If so, where? Do they correspond in size and shape with the valves of the shell? Where is the mantle attached to the shell? Where to the body? What is its texture? Color? Do you find any variations in either? To what are such variations due? Notice a thickened band, the **pallial muscle**, near the margin.

What is the texture of the extreme margin of the mantle? Color? Structure? Notice on the dorsal surface, in front of the posterior adductor muscle, a thin portion of the mantle. This portion covers the **pericardial chamber**.

- c. The **adductor muscles of the valves**.—How many are there? How are they situated? How are they distinguished from the rest of the body? What is the shape and size of each? Do they differ in shape and size? How are they attached to the shell? Are they covered by the mantle? How do you account for their number? In what direction do their fibres run? Why do they run in this direction? Why are these muscles called “adductors”? What advantage in having them in the position in which you find them?

At the upper end of each adductor muscle look for

- d. The **retractor pedis muscles**.—Compare with c in every respect. Do these muscles form a “scar” on the shell?

A little below the anterior retractor muscle find

- e. The **protractor pedis muscle**.—What is its shape? Size? Compare with the adductor and retractor pedis muscles in every respect.

At the posterior end of the body find

- f. The **siphons**.—How many are there? How are they formed? What is their relative position to each other? How are they separated? How long are they? How wide? With a pipette inject water into each siphonal opening. Where does the cavity of each lead? Note the **tentacles**—their number, shape, size, color, and structure.



Turn back one of the lobes of the mantle and note

- g.* The **pallial cavity**.—How is it formed? What does it contain? Note its division into a large ventral cavity, the **branchial cavity**, and a much smaller dorsal cavity, the hinder portion forming the **cloacal cavity**.
- h.* The **abdomen**.—What is its relation to the muscles? What is its shape? Color? Is it bilaterally symmetrical? Is there a head? Are there any bones in the body? How is it held in the shell?  
Projecting from the lower margin of the abdomen is
- i.* The **foot**.—What is its position with regard to the abdomen? To the pallial lobes? To the gills? What is its shape? Size as compared with the abdomen? Color? Structure? To what is it attached? In what direction does it project?
- j.* The **gills** or **gill-plates**.—What is their position with regard to abdomen, foot, siphons, and mantle? Number? Shape? Size as compared with the mantle folds? Color? How attached to the body proper and the other parts? Cut along the line of attachment of the outer gill to the mantle. Notice that a canal, the **supra-branchial** or **cloacal chamber**, is thus laid open. Notice also running down into the gill between its two faces a number of fine tubes, the **water-tubes**, separated from one another by partitions. These tubes are closed at the lower margin of the gill, as may be proved by passing a fine bristle into a tube. Carefully split open a gill, and examine both surfaces with a hand-lens. Note that each

gill consists of two lamellæ united by the partitions mentioned above, which are the **inter-lamellar junctions**. Examine also with the low power, and try to make out the oval **inhalent apertures** opening on the face of the gill into the water-tubes. Scrape the surface of a gill of a living mussel, and examine the ciliated cells thus set free. Examine a piece of a split gill of a living mussel under the low power, and note the **ciliary action**. Compare the gills with each other as regards shape, size, color, structure, attachment, etc.

Make drawings showing the gross and the minute structure of a gill.

k. The **mouth**.—What is its position with regard to foot, anterior adductor muscle, siphons, and gills? What is its shape? Size? Are there any jaws? Teeth? A tongue? What kind of food can the mussel eat?

l. The **labial palpi**.—What is their position with regard to the mouth and gills? Number? Shape? Color? Attachments? Compare with gills as regards shape, size, and structure. Are they at all like the palpi seen on the lobster and locust?

m. The **anus**.—What is its position? With which siphon is it connected?

Make the following drawings: (1) A side view of the mussel lying in one valve of the shell, to show the position of the muscles, etc.; (2) a side view with one mantle lobe turned back; (3) a side view with mantle lobe and gills turned back.

*Internal Anatomy.**A.—The Digestive System.*

Using an alcoholic specimen, push a guarded bristle into the mouth, and with scissors cut open the alimentary canal from the mouth to the stomach, using the bristle as a guide. Then in a similar manner trace the course of the intestine backwards from the anus. If necessary, the alimentary canal may be injected through the anus with a mixture of equal parts of plaster of Paris and water. The mixture must be strained through fine cloth before being used.

- a.* The **mouth**.—This has already been studied.
- b.* The **oesophagus**.—What is its relation to the mouth as regards diameter and the direction in which it runs? To the anterior adductor muscle? What is the nature of its walls?
- c.* The **stomach**.—How is it distinguished from the oesophagus? What is its position with regard to the hinge-ligament?  
Find surrounding the stomach and oesophagus a dark-colored mass,
- d.* The **liver**.—What is its relation to the anterior adductor muscle? Look for the opening of the **bile-duct** into the oesophagus anterior to the opening of the latter into the stomach, and into the stomach itself.
- e.* The **intestine**.—Into what part of the stomach does it open? Trace it through the yellowish-white **generative gland**, by picking away the latter with the fine forceps. Does it enter the substance of the foot? What is its course through

the abdomen of the animal? What is the relation of its posterior end, the **rectum**, to the hinge-ligament? To the posterior adductor muscle? Notice that the rectum runs through the pericardial cavity. Into what does the anus open? Notice the prominent ridge or **typhlosole** on the ventral side of the rectum. Where is this ridge largest? How does this compare in position and structure with the typhlosole of the earthworm?

Draw a diagrammatic longitudinal section of the body, showing the course of the digestive system.

*B.—The Circulatory System.*

Remove from the shell a living mussel in water, being very careful not to injure the soft parts, and note the beating of the heart as seen through the pericardial wall. Study the number of beats per minute and the movements of the heart. Open the **pericardium**, noting the thickness of its wall and the shape and extent of the pericardial cavity. If living specimens cannot be had, the heart may be found by cutting the upper edge of the upper siphon forward to the umbo. Notice the **heart**, consisting of a median **ventricle** and two lateral fan-shaped **auricles**.

- a. The **ventricle** (wrapped around the intestine).—What is its position in the pericardium? What is its shape? Size? Color? Are its walls firm or flexible? Trace from the ventricle an **anterior aorta** on the upper side, and a **posterior aorta** on the lower side of the intestine.
- b. The **auricles**.—What is their relation to the ventricle? What is the structure of their walls?

How do they communicate with the ventricle? What is the shape of their cavity? What is their relation to the gills? Look for veins running along the upper edges of the gills and communicating with the auricles.

*C.—The Nervous System.*

Using an alcoholic specimen, and dissecting in a dish of fifty per cent. alcohol, endeavor to make out the following parts:

- a. **The cerebral ganglia.** — Separate the palpi on each side of the mouth, and just under the skin, between their bases, the ganglia will be found. What is the position of the ganglia with regard to the mouth? How many are there? What is their shape? Size? Color? In what direction do nerves pass off? Look for nerves passing to the anterior adductor muscle; to the palpi; to the mantle lobes; to the foot, connecting with the **pedal ganglia**, and hence called **cerebro-pedal connectives**; to the **parieto-splanchnic ganglia** at the posterior end of the body, and forming the **cerebro-splanchnic connectives**, and from one cerebral ganglion across over the mouth to the other ganglion, the **inter-cerebral commissure**. Do you find that these ganglia are bilaterally symmetrical as regards position and branches?
- b. **The parieto-splanchnic or visceral ganglia.** — To see these, separate the gills at the posterior end of the body. Compare these ganglia with the cerebral ganglia in all points of structure. Endeavor to make out nerves running to the

posterior adductor muscle, to the gills, and to the pallial lobes and connectives, already partially traced, running to the cerebral ganglia.

- c. The **pedal ganglia**.—Very careful dissection will be needed to show these. They lie in the middle line of the foot, close to the point where the foot and the abdomen join, and about one-third of the length of the former organ from its anterior end. Compare these ganglia with the others studied, and try to trace the connectives running to the cerebral ganglia. Look for the pair of **otocysts**, or "ear," a short distance behind and below the pedal ganglia.

Make a diagram showing the position of all of the ganglia and the course of the nerves.

*D.—The Excretory System.*

Lying under the pericardium, extending forward from the posterior adductor muscle, find on each side a wide, dark-colored tube with spongy walls. Endeavor to make out the lower portion of the tube with its folded spongy walls, the "**kidney**," or **organ of Bojanus**, and a thin-walled conducting tube, the "**ureter**." Look for the opening of the latter into the anterior end of the pericardium. Pass a bristle from the pericardium into the organ of Bojanus. Opening into the cloacal chamber immediately below and behind the anterior end of the pericardium look for a small pore, the **renal aperture** or external orifice of the "kidney." Near the renal aperture look for the **genital aperture**.

*E.—The Examination of Transverse Sections.*

Wedge open the valves of living specimens and put them into one per cent. chromic acid for about two days, then transfer to seventy per cent. and ninety per cent. alcohol for one day each. Remove the body from the shell, being careful to disturb the parts as little as possible. Place the mussel on a board, and with a razor cut the entire body into a series of parallel transverse sections, about a quarter of an inch thick. Float these sections in order in a long dissecting-pan containing fifty per cent. alcohol, and study the relation of the various organs.

Draw the following sections: (1) Through the stomach, (2) through the heart, (3) through the middle of the posterior adductor muscle.

As regards structure, which of the animals that you have studied does the mussel most closely resemble?

*Habits.*

A number of interesting observations on the habits of the fresh-water mussel may easily be made by placing specimens in an aquarium or in tubs or pans having the bottom covered with three or four inches of sand or mud. By changing the water from time to time the animals may be kept alive for weeks. Study the position of the animal in the water, the manner in which the foot is used as an organ of locomotion, watch the water flowing into and out of the siphons by scattering particles of indigo near the orifices, test the sensibility of the siphonal tentacles by gently touching them with a bristle, etc.

For comparative work use the clam (*Mya* or *Venus*) or the oyster (*Ostrea*).

### **Frog (*Rana Sp.*)**

*Material.*—Almost any creek, pond, or marsh will furnish an abundance of frogs during the warm season, but especially in the spring. They should be caught uninjured. Maimed and mutilated specimens are of little value for anatomical purposes, to say nothing of the cruelty practised in capturing them with sticks, stones, and spears. City students may be supplied by fish-dealers, some of whom are usually acquainted with the men who supply the markets with frogs' legs. The frogs may be kept in a deep box covered with wire netting, and containing several sods, which should frequently be watered to keep the grass in good condition. At one end of the box may be placed a pan—a dripping-pan will answer—filled with water, in which the frogs may swim. The box should stand in a dimly lighted, cool place, as in a cellar, and the water should be changed every few days.

If the frogs vary much in size, the large ones should be kept separate from the smaller; otherwise the latter will be eaten. In a properly prepared box specimens may be kept all winter, with no other care than changing the water in the pan and moistening the sods. It is almost, if not quite, impossible to get the creatures to eat anything besides their smaller companions, no matter how tempting may be their food. Should some of them be frozen in the pan, let them thaw slowly, and they will be in as good condition as ever.



Both living and alcoholic material should be used. The latter will dissect more easily than the freshly killed specimens. Frogs may be killed in a few minutes by being placed in a covered bowl with a wad of cotton or a piece of cloth, upon either of which a few drops of ether or chloroform have been poured. To preserve them, open the body by cutting along the median ventral line from the fore to the hind limbs. Open the skull and the spinal canal by cutting away the bone with scissors. Place the body in seventy per cent. alcohol for two or three days (being careful to see that the abdominal cavity is kept open, so that the alcohol may have access to the internal organs), then transfer for a day or two to stronger alcohol.

Two skeletons should be prepared, one having the bones separated, the other having them connected by the natural ligaments. The first one may be prepared by cutting off the bones as much as possible of the flesh of a fresh or alcoholic specimen, and soaking it for several days in water or in water to which has been added just enough potash to give the solution a decidedly slippery feel. Warmth will hasten the process. When sufficiently macerated, the flesh may be removed from the bones with a tooth-brush. If it be desirable to keep the bones of the fore and hind feet separate, wrap each of those organs in a piece of fine cloth. After being cleaned the bones may be bleached by laying them in a sunny window where they will not be blown away. The second skeleton should be made from a freshly killed frog, though an alcoholic specimen will do. Cut off the flesh, being very careful not to trim the ligaments too closely at the joints, nor to cut away the cartilaginous portion of the skeleton, especially that of the hyoid apparatus. Throw the preparation into Wickersheimer's fluid for a

week or two, then cut away all of the superfluous ends of muscles, ligaments, etc., leaving just enough to hold the bones together. In this preparation the membranous parts will be found to be so flexible as to permit of all the natural motions of the joints. Specimens prepared in this way may be exposed to the air for several years without deterioration. Should the ligaments become stiffened, a few days' soaking in the fluid will soften them again.

The instruments and reagents to be provided include a medium-size and a small scalpel, scissors, medium-size and fine forceps, lens, pipette, bristles, blow-pipe, fifty per cent. alcohol, seventy-five per cent. alcohol, watch-glass, dissecting-pan, dilute nitric or hydrochloric acid, magenta, normal salt solution, one-half per cent. solution of silver nitrate, distilled water, hæmatoxylin, glycerine, chloroform, eosin, iodine, and microscope.

*Method of Examination.*—Study alcoholic specimens in dissecting-pans containing fifty per cent. alcohol. Recently killed frogs may also be examined advantageously in alcohol. While working out the gross anatomy and physiology, let the student have in front of him a jar of water containing a living frog.

#### MORPHOLOGY

##### *External Anatomy.*

- a. *Shape.*—What is the shape of the body as a whole? Does it possess well-marked dorsal and ventral surfaces? If so, how are they distinguished? What differences between the shape of the anterior and posterior ends? Examine several

frogs to find decided variations in shape. Is the shape of the body adapted to the frog's mode of life?

- b. Size.*—What is the length of your specimen? Width? Where is the place of greatest width? Of least? Where that of greatest depth? Least?
- c. Color.*—What is the general color of the body? Do you find decided differences between the colors of the dorsal and ventral surfaces? What colors do you find on the dorsal surface? How are they arranged? Is the disposition of colors on the right the same as that on the left side? Examine a number of frogs and see whether or not they are colored alike. Do all show the same arrangement and the same shades of color? Can you give any reasons for the presence and distribution of the colors found? If practicable, examine the same frog at different seasons of the year to see whether or not there be any variation in the color of a single individual.
- d. General Structure.*—Note that the entire body consists of an **axial portion**, composed of **head** and **trunk**; and an **appendicular portion**, the **limbs**. Study the skin covering the body. What is its texture? Is it closely fastened to the underlying parts? Does it vary in this respect? Does it bear any outgrowths, as scales, hairs, feathers, etc.?
1. The **head**. — What is its shape as seen from above? From the side? Is it connected to the body by a neck? How do you distinguish

the boundary line between head and body? How much of the axial portion of the body does the head form? Where is the widest part of the head? What is the shape of its anterior end? Where is the mouth? What is its shape? In which direction does it open? How far around on the sides of the head does the mouth extend? Compare in position, shape, size, mode of opening, etc., with the mouth of the lobster. Note the **eyes**.—What is their position? Shape? Color? Are there **eye-lids**? If so, how many? How do they differ in shape, size, color, texture, mobility, etc.? Compare with the lobster. How do you account for the position and prominence of the eyes? Can they in the live frog be depressed and raised in the sockets? If so, give reasons? Look for the **brow-spot** on a line connecting the anterior borders of the eyes. Anterior to the eyes find the **external nares** or **nostrils**. Study their position, size, and shape. What is the texture of the skin surrounding them? Can they be closed? What reasons can you give for the position of the nostrils? Posterior to the eyes find the **tympanic membranes**. What is their shape? Size? Color? What is the texture of the membrane? How is it supported? Is it tightly or loosely stretched?

How many *paired* openings does the head bear? How many *unpaired*? What are they in each case?

2. The **trunk**.—What is its shape? How does the dorsal differ from the ventral surface in shape? Compress the trunk from side to side between

the thumb and fingers. Where do you feel the hard parts of the skeleton? Note the "hump" in the back of a living frog which is sitting naturally. Note also the ridge, the **urostyle**, running backward from the "hump," and ending near the posterior end of the trunk. Find at the posterior end the **cloacal aperture**. What is its exact position? Shape? What is its position with regard to the end of the **urostyle**?

3. The **limbs**.—Study first the anterior limbs. To what part of the body are they attached? In which direction do they project? What is their shape? Are they long enough to reach to the tip of the head? What is their diameter? Does it vary greatly at any point? Make out the following regions on each limb: the **brachium** or upper arm, the **antebrachium** or fore arm, and the **manus** or hand. What portion of the entire limb does each of these regions form? In which direction does each point? Compare the **brachium** and **antebrachium** with regard to length. How many **digits** or fingers on the **manus**? Is the **pollex** or thumb present? Of how many joints does each digit consist? Do you find a web between the adjacent digits? Do they differ at all with regard to the thickness of the skin covering them? Do they bear nails or claws?

Study the hind limb, and make out the following regions: the **femur** or thigh, the **crus** or leg, and the **pes** or foot. Compare as above all of the regions with one another and with the corresponding regions of the fore limb.

What are the main points of resemblance? What are the principal differences? How do you explain the latter? Do you find a **hallux** or great toe? In what respects does the skin of the thigh differ from that on other parts of the body?

Make diagrams illustrating the main features of the external anatomy.

In what respects, if any, do the appendages of the frog resemble those of the lobster and the locust?

### *Internal Anatomy.*

#### *A. The skeleton.*

Study the articulated skeleton prepared in Wickersheimer's fluid, using for comparison the individual bones of the other skeleton. Note the **axial skeleton**, consisting of the **skull** and **vertebral column** or "backbone," and the **appendicular skeleton**, composed of the **limbs**, which are more or less closely attached to the axis by means of the **limb-girdles**; the anterior being the **pectoral-girdle** or **shoulder-girdle**, the posterior the **pelvic-girdle** or **hip-girdle**.

- a. The **vertebral column**.—What is its general appearance? Is it a single bone? If not, why is it called the "backbone"? Of how many parts does it consist? Is the vertebral column hollow or solid? Notice that the entire column may be divided into two regions, an anterior and a posterior, the latter formed of the **urostyle**. What is the shape of the urostyle? Structure? How does its length compare with that of the anterior portion of the column? Is it a single piece, or does it consist of segments? To what is its an-

terior end attached? Posterior end? Is it solid or hollow? Note the prominent ridge. On what part of the urostyle is it found? What is its shape? Near the anterior end and on each side find a small hole or **foramen**, through which nerves enter the urostyle. Draw the urostyle as seen from the side.

Examine the anterior portion of the vertebral column. Of how many segments or **vertebræ** does it consist? Are the **vertebræ** quite alike in general appearance? Examine a single **vertebra**, *e.g.*, the third or fourth, and note that it is a bony ring which bears several projections or **processes**. The ventral portion of the ring is the **centrum** or **body**. What is its shape as seen sidewise? What is the shape of its ends? Is it composed of dense or of spongy bone? On the dorsal side of the centrum is the **neural arch**. How is it formed? What is its shape as seen from the end? On the dorsal side of the arch find a single projection, the **spinous process**. What is its shape? Length? In which direction does it point? On each side of the neural arch find a **transverse process**. How do these processes compare in shape, size, and structure with the spinous process? In which direction do they extend? Why called "transverse"? Are they attached to any other bones? Find also a pair of **anterior articular** and a pair of **posterior articular processes**. What is the position of each pair? Compare in all respects with the transverse processes. Why are they called "articular" processes? Do you find any ribs? Draw the vertebra as seen from the end, from the side,

and from above. Compare all of the other vertebræ with the one just studied, noting especially the first or **atlas** and the last or **sacrum**. Does the former have a spinous process? Transverse processes? Anterior articular processes? Posterior? Note the large space left between the roof of the neural arch and the base of the skull. Look for the projection, the **odontoid process**, on the centrum of the atlas. What is its shape? With what does it connect? Draw the atlas. What modification has taken place in the transverse processes of the sacrum? To what are they attached? Draw the sacrum. Examine two adjacent vertebræ, and note how the articular processes of the one move upon the articular processes of the other. Examine an entire vertebral column, and note the row of openings, the **inter-vertebral foramina**, on each side of the vertebral column, immediately below the articular processes. How are these openings formed? Compare these with the foramina found on the urostyle. Have you any evidence that the urostyle is made of more than one piece? Is the segmental portion of the vertebral column of a living or of a recently killed frog very flexible? If so, in which direction does it bend?

- b. The **skull**.—What is its shape as seen from above? From below? From the side? Notice the large **eye-socket** on each side of the **cranium**. Back of each eye-socket find a tubular portion, the **auditory capsule**. What is its position with relation to the cranium? To the tympanum? At the anterior end of the cranium find on each side



an **olfactory capsule**. Examine the lower jaw, the **mandible** or **inferior maxilla**. What is its shape? What is its position when the mouth is closed? Does it bear teeth? Are its two sides immovably connected? Of how many parts is each half of the mandible composed? On its upper edge find a groove in which lies **Meckel's cartilage**. Where does this cartilage begin? How far does it extend? Do you find any foramina in the lower jaw? If so, where are they? Draw the mandible as seen from above. Notice that the upper part of the skull consists of a system of parts attached to the cranium and the **sense capsules**. Examine the posterior end of the skull and find a large opening, the **foramen magnum**. What is its shape? Size? What is its relation to the **neural canal** in the spinal column? To the **cranial cavity**? Find a convex tubercle, the **occipital condyle**, on each side of the foramen magnum. What is the shape of the condyle? What is the direction of its longer axis? The nature of its surface? Its relation to the atlas? Each condyle is borne upon one of the **exoccipital bones**. What is the relation of these bones to the foramen magnum? What is their shape? Do the exoccipital bones form any part of the auditory capsules? Note that each condyle is pierced by a foramen which is the exit of the tenth cranial nerve (**vagus**). Notice on each side the ridge along which is the junction between the exoccipital bone and the **pro-otic bone**. What is the shape of the latter? What part of the auditory capsule does it form? Is any part of the front of the

auditory capsule cartilaginous? If so, what is the relation of this part to the pro-otic bone? Attached to the outer edge of the pro-otic and extending downward to the posterior end of the upper jaw find the **squamosal bone**. What is its shape? What is its relation to the tympanum? Examine the cartilaginous ring, the **tympanic ring**, upon which the tympanum is stretched. What is the relation of the ring to the squamosal bones attached to the under surface of the tympanum? Find the **columella auris**. What is its structure? Shape? To what part of the tympanum is it fastened? Trace the columella to its inner end, which closes the opening or **fenestra ovalis** in the auditory capsule.

In carefully prepared skeletons look for a cartilaginous rod, the **styloid cartilage**, running from the pro-otic to the **hyoid bone**. Examine the roof of the skull and find a pair of bones, **fronto-parietals**, extending forward from the exoccipitals and pro-otics. What is their shape? How far forward do they extend? What is their relation to the cranial cavity? Examine the **sagittal suture**, along which these bones are united.

At the anterior end of the fronto-parietals look for the **sphenethmoid** or **girdle bone**. Anterior to the latter is a cartilaginous structure on each side of which is a **nasal bone**. What is its shape? In which direction does it extend? What is its position with regard to the fronto-parietal? How is it connected to the latter? How does it differ structurally from the sphenethmoid? In front of the nasal bones lie the

two **pre-maxillæ**. What is their shape? Note the **ascending process** which each bears. What is the direction of the process? How are the pre-maxillary bones united to each other? To the nasal bones? Extending backward from the lower end of each pre-maxilla find the **maxillary bone**. How far back can you trace it? What is its shape? What is the shape of the lower edge? Note the **teeth**. On what part of the maxilla are they borne? How are they arranged? What is their shape? Do they vary in shape and size? How are the maxilla and the pre-maxilla of the same side connected? Do you find teeth on the pre-maxilla? At the posterior end of the maxilla find the **quadrato-jugal bone**. To what part of the skull is it attached? Lying ventrally from the squamosal, and towards the median line from the quadrato-jugal, find the **pterygoid bone**, composed of three branching projections. With what does each projection articulate?

Examine the under side of the skull, and find the **parasphenoid bone**, forming the floor of the cranial cavity. What is the shape of this bone? What is its relation to the auditory capsules? Find between this bone and the fronto-parietal of each side a foramen for the exit of the **second cranial or optic nerve**. Examine the ventral and lateral portions of the sphenethmoid, the dorsal portion having been previously studied. What is the shape of this bone? Structure? Is the cranial cavity entirely enclosed by bony structures? Does the cranium show any trace of segmentation? Compare in this respect with

other parts of the axial skeleton. Directly below the nasal bone find the **palatine bone**. What is its shape? Relation to the sphenethmoid? To the maxilla? What is the direction of the palatine bone with regard to the cranial axis? In front of the palatines find the **vomers**. Examine their shape, structure, and articulations. Study the position and structure of the **vomerine teeth**.

Study the position, shape, structure, and attachments of the **hyoid apparatus**. If the parts are not found on the prepared skeleton, they may be dissected out on an alcoholic specimen.

Examine a vertical longitudinal section of the skull, made a little to one side of the median line, and note the position of the various parts. Note especially the **septum nasi**, which separates the nasal chambers, and the foramen, through which the **first cranial or olfactory nerve** passes.

Review all of the bones of the skull, making a list of those which enclose the cranial cavity, the auditory capsule, and the olfactory capsule. Make also a list of those which form the jaws.

- c. The **pectoral girdle**.—What is its position? Is it a complete girdle? How is it attached to the axial skeleton? Notice that each half of it can be divided into two portions, the **scapular portion**, extending dorsally from the shoulder-joint, and the **coracoid portion**, extending ventrally. Is the scapular portion of the right directly connected with that of the left side? Compare with the coracoid portions. Examine the two parts of

the scapular portion, the **supra-scapula** or cartilaginous part, and the **scapula** or bony part. What is the shape of the supra-scapula? Is it entirely cartilaginous? Is it attached by a movable or by an immovable connection to the scapula? Draw the supra-scapula. What is the shape of the scapula? Notice the depression, forming a portion of the **glenoid cavity**, at the ventral end of the scapula. What occupies this depression? Draw the scapula. Study the two parts of the coracoid portion of the girdle, the posterior part or **coracoid bone**, and the anterior part or **clavicle**. What is the shape of the coracoid? Does any part of it help to form the glenoid cavity? Draw the coracoid bone. What is the shape of the clavicle? How is it attached to the scapula? Does the clavicle unite directly with the scapula? Between the coracoid and the clavicle is the **coracoid foramen**. How is it formed? What is its shape? Examine the **sternum**, consisting of the following parts named from before backward: **episternum**, **omosternum**, **epicoracoids**, **sternum proper**, and **xiphisternum**. Locate first the epicoracoids, a pair of narrow cartilages lying between the inner ends of the coracoids and clavicles. Posterior to the inner ends of the coracoids lies the sternum proper. What is its shape? Length? Is it bone or cartilage? Draw. At the posterior end of the sternum find the xiphisternum. Of what is it composed? What is its shape? Draw. In front of the clavicles find the omosternum. Is it attached to the clavicles? What is its shape? Compare with the sternum proper. Draw. Attached to

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the anterior end of the omosternum find the episternum. Compare in all respects with the xiphi-sternum. Draw the episternum. Is the ventral portion of the pectoral girdle flexible? Does the girdle as a whole permit of a very great range of motion? Of how many pairs of bones does the pectoral girdle consist? Pairs of cartilages? Of how many median bones? Median cartilages?

- d. The **fore limb**.—Examine the right fore limb, and note its division into arm or **brachium**, forearm or **antebrachium**, wrist or **carpus**, and hand or **manus**. Examine the arm-bone or **humerus**. What is its shape? How is it connected to the pectoral girdle? Note the enlarged upper end or **head** of the humerus. How does its surface differ from that of the rest of the bone? Extending downward from the head find the **deltoid ridge**. On what part of the humerus is it? How is it formed? How far does it extend? Do you find any other ridges on this bone? Examine the skeletons of several frogs of the same size. Are the ridges equally prominent in all? How does the lower differ from the upper end of the humerus? In how many directions may the humerus be moved? Why? Compare with your own shoulder-joint. Draw the humerus.

Examine the bone, the **radio-ulna**, of the forearm. What is its shape? Does it appear to be composed of two consolidated bones? What is the shape of the upper end of the radio-ulna? Which is the **radius** and which the **ulna**? Study

the manner in which the radio-ulna articulates with the humerus. Note the **olecranon process** back of the elbow-joint. In how many directions may the forearm be moved? Compare with the humerus and with your own forearm. Draw the bone.

Study the bones in the wrist, using a lens if necessary. How many carpal bones are there? How are they arranged? Of how many motions is the frog's wrist capable? Compare with those of your own wrist. How do you account for the differences? What is the natural position of the hand of a living frog? How many digits do you find in the skeleton of the hand? Compare with the number visible in the hand of the living frog. Examine the first bone or **metacarpal** in one of the digits. What is its shape? Compare all of the metacarpals. What differences do you notice? Notice that, in addition to the metacarpal, each digit consists of a number of smaller bones, the **phalanges**. How many do you find in each digit? What variations in shape and size of the phalanges in a single digit? Does the thumb or **pollex** differ in structure from the other digits? Of what motions are the digits capable? Draw the frog's hand. Compare the structure of the frog's hand with your own. What important skeletal differences can you distinguish?

- e. The **pelvic girdle**.—What is its shape? In which direction does it extend? Is it attached to the axial skeleton more or less firmly than the pectoral girdle? What is its relation to the urostyle? Examine the left half, and endeavor to make out

that it consists of three consolidated bones, (1) the **ilium**, running nearly parallel to the urostyle, and forming with its posterior end more than half of the anterior portion of the disk-like mass lying between the heads of the thigh-bones; (2) the **ischium**, forming the greater part of the posterior half of the disk-like mass; and (3) the **pubis**, forming the ventral portion of the disk. What is the shape of the ilium? Note the prominent **iliac crest**. How far does it extend? Where is it widest? How is the ilium attached to the sacrum? Note the **acetabulum**, into which the head of the thigh-bone fits. How is it formed? What is its shape? Diameter? What is the shape of the ischium? Of the pubis? Make a drawing showing a side view of the left side of the pelvic girdle.

- f. The **hind limb**.—Compare the thigh-bone or **femur** with the humerus. What differences do you find? Note the **nutritive foramen**, near the middle of the shaft of the femur. What range of motion has the femur? Compare the acetabulum with the glenoid cavity. Draw the femur. Compare the bone, the **tibio-fibula**, of the leg or **crus** with the bone of the forearm, and note the resemblances and differences. Draw. Compare the knee-joint with that of the elbow as to structure and range of motion. Note that the ankle or **tarsus** consists of two rows of bones, of which two bones, the **astragalus** on the inside and **calcaneum** on the outside, unite with the tibio-fibula and several other smaller bones. Compare the other tarsal and the metatarsal



bones and the phalanges with the corresponding parts of the fore limb. Look for other nutritive foramina in the different bones of the hind limb.

What resemblances and differences can you trace between the arrangement and structure of the frog's skeleton and that of the lobster? Between the motions allowed by the joints? Compare the frog's with a human skeleton or with pictures of the same. Does the frog have a **pa-tella** or "knee-cap"? Is there a corresponding bone at the elbow?

*B.—The Muscular System.*

Examine the muscles of an alcoholic specimen in a pan containing fifty per cent. alcohol. Keep the specimen moist all the time. Remove the skin from the entire body, noting the threads and bands of **connective tissue** which bind the skin to the underlying parts. In what regions is the skin loosely attached? At what points is it most closely fastened? Can you give any reasons for this arrangement of the skin? Wash away any coagulated substance, the **lymph**, which may be found lying in the depressions between the muscles, and trim away the ragged ends of connective tissue. Note the groups of muscles in the various regions. What distinctions can you give between those of the abdomen and those of the limbs? What is the color of the muscles? Are any of them so thin as to be almost transparent? If so, where are they? How do you distinguish one muscle from another? In studying each muscle, separate it carefully from its

neighbors by cutting the connective tissue which binds them together, and endeavor to make out its shape, the direction of its fibres, its origin, its attachment, and the motion which it produces. Use the articulated skeleton for comparison. Make the drawings necessary to show the position and arrangement of the parts. This may usually be done by drawing on one side of the body the superficial, and on the other side the deeper muscles.

Only the more important muscles are given. Examine them in the following order :

I. *The muscles of the trunk.*

Pin the frog down on its back, and notice that a certain group of muscles covers the *ventral side* of the body.

- a. The **pectoralis**, fan-shaped, running from the sternum to the shoulder, and consisting of several parts. Where does each part originate? Find one part extending down upon the abdomen. Are all of the parts inserted at the same point?
- b. The **rectus abdominis**, running from the pubis. Note the **linea alba**, which separates the two recti. Of what is the linea composed? Note also the transverse division of each rectus into parts or **bellies**. How many such parts are there? What is the relation of the anterior end of this muscle to the pectoralis?

Remove the two muscles just examined and find

- c. The **oblique muscles**, covering the sides of the abdomen. The **obliquus externus**, with

fibres running downward and backward from the dorsal surface, and the **obliquus internus**, with fibres running downward and forward.

Turn the frog over, and study the muscles of the *dorsal surface*.

- d.* The **depressor mandibuli**, a triangular muscle, posterior to the tympanic membrane.
- e.* The **latissimus dorsi**, a triangular muscle posterior to the depressor.
- f.* The **infra-spinatus**, partly covered by the latissimus. Raise the supra-scapula, and examine the muscles which attach it to the body.  
Clear away the transparent membrane or **aponeurosis** covering the back, and below it find
- g.* The **extensor dorsi communis**, running from the urostyle to the head. Lying under this muscle look for short muscles connecting the transverse processes of the vertebræ.
- h.* The **gluteus**, arising from the outer side of the posterior portion of the ilium, and running backward.

Make lists of all the muscles whose action would cause the body to bend (1) downward, (2) upward, (3) sidewise; (4) would move the head upward; (5) would depress the lower jaw; (6) would move the anterior limbs; (7) would move the posterior limbs.

## II. *Muscles of the head.*

On the *ventral surface* of the head find

- a.* The **mylohyoid**, lying directly beneath the skin.  
Remove the mylohyoid and find
- b.* The **geniohyoid**, to one side of the median line.

Notice that posteriorly it divides into two portions.

- c. The **sternohyoid**, its anterior end lying between the divisions of the geniohyoid. What is the relation of the sternohyoid to the rectus abdominis?
- d. The **hyoglossus**, uniting in the median line with its fellow, and partly covered by the geniohyoid. Study the relation of this muscle to the tongue.
- e. The **submentalis**, at the anterior angle of the lower jaw.
- f. The **petrohyoids**, four small muscles running from the auditory capsule to the hyoid bone.  
On the *side* of the head find
- g. The **masseter**, lying immediately in front of the union of the upper and lower jaws.
- h. The **temporalis**, running down between the eye and the auditory capsule.
- i. The **pterygoideus**, under the temporalis.

Make lists of the muscles which (1) increase the size of the cavity of the mouth; (2) move the tongue; (3) raise the hyoid bone; (4) raise the lower jaw.

Carefully cut away the three muscles mentioned above, lay the frog on its back, remove the lower jaw and the mucous membrane of the roof of the mouth, and find the *eye muscles*, consisting of

- j. The **levator bulbi**, a sheet of muscle lying between the eyeball and the mucous membrane. What important use has this muscle?

Remove the levator bulbi and look for

- k. The **recti muscles**, four small muscles arising together from the inner posterior angle of the eye-socket.
- l. The **obliqui muscles**, two muscles arising from the anterior end of the eye-socket.
- m. The **retractor bulbi**, a cone-shape muscle, arising from the angle of the parasphenoid. What is the function of this muscle?

Make a list of the muscles which (1) raise or protrude the eyeball; (2) depress or lower it; (3) rotate it in the socket.

### III. *Muscles of the front limb.*

- a. The **sternoradialis**, a triangular muscle lying in front of the shoulder-joint. To what muscle in the human body does it correspond?
- b. The **deltoid**, a narrow muscle lying in front of the sternoradialis.
- c. The **triceps brachii**, on the upper surface of the arm.
- d. The **flexor muscles** of the forearm and digits, covering the inner side of the antebrachium and lower surface of the manus.
- e. The **extensor muscles** of the forearm and digits, lying on the outer side of the former and upper surface of the latter.

Which of these muscles will draw the arm forward? Which bend it? Which straighten it? What relation has the deltoid ridge of the humerus to the attachment of muscles?

IV. *Muscles of the hind limb.*

Straighten the hind leg to its fullest extent, lay the frog on its back, then examine the muscles on the *ventral side of the thigh*.

- a. The **sartorius**, a narrow, band-like muscle crossing the thigh obliquely.
- b. The **adductor magnus**, lying towards the median line from the sartorius, and partly covered by the latter.
- c. The **adductor longus**, on the outer edge of and partly covered by the sartorius.
- d. The **rectus internus major** and the **rectus internus minor**, two muscles forming the inner border of the thigh.

Lay the frog on its ventral surface, and on the *dorsal side of the thigh* find

- e. The **triceps extensor femoris**, forming the outer edge of the thigh, and consisting above of three parts, of which one, the **rectus anticus femoris**, forms one side of the angle between the side of the abdomen and the thigh, and another, the **vastus externus**, appears about midway between the first-named muscle and the posterior end of the urostyle.
- f. The **semimembranosus**, lying towards the median line from the vastus externus, and marked by a tendon running obliquely across the muscle.
- g. The **biceps femoris**, lying between the vastus externus and the semimembranosus.
- h. The **pyriformis**, passing down between the upper ends of the biceps and the semimembrano-

sus. Separate the rectus internus and the adductor magnus, and find between them

- i. The **semitendinosus**, a muscle with two heads.

Divide the sartorius transversely and find

- j. The **adductor brevis**, whose head lies between the sartorius and the adductor magnus.

- k. The **pectineus**, lying along the outer margin of the adductor brevis.

Push aside the **vastus internus** and find

- l. The **ilio-psoas**, lying dorsally from the adductor brevis.

- m. The **quadratus femoris**, posterior to the gluteus.

- n. The **obturator**, towards the median line from the quadratus femoris.

Which of the above-named muscles pull (*flex*) the thigh? Which draw (*extend*) it backward?

On the *leg* find

- o. The **gastrocnemius**, the muscle of the calf of the leg. Note its large tendon, the **tendo Achillis**.

- p. The **tibialis anticus**, along the outer margin of the leg.

- q. The **tibialis posticus**, lying along the inner margin of the gastrocnemius.

- r. The **peroneus**, lying between the tibialis anticus and the gastrocnemius.

- s. The **extensor cruris brevis**, along the anterior margin of the tibialis anticus.

Examine the muscles and tendons of both surfaces of the foot.

Which of the leg muscles are extensors? Flex-

ors? Which muscles flex the foot? Extend the foot? What reasons can you give for the large size of the thigh muscles? Of the gastrocnemius? For the great difference in size between the muscles of the anterior and those of the posterior limb? When the frog's leg is flexed upon the thigh, does it lie in the same position as the human leg?

Compare the arrangement of the principal muscles of the frog with the corresponding muscles of the human body, as shown on charts, anatomical plates or on a manikin.

*C.—The Digestive System.*

*I. The mouth-cavity.*

Pull down the lower jaw and open the mouth to its widest extent.

What is the shape of the mouth-cavity? Can it be enlarged? If so, in what directions? What external organs mark the posterior boundary of the mouth-cavity? Are there any lips? What is the color and texture of the mucous membrane lining the cavity? Can the position of the eyes be determined by an examination of the roof of the mouth? Press upon the external surface of the eyeball. What change takes place in the mucous membrane of the roof of the mouth?

Examine the roof of the mouth and find the following structures:

- a.* The **maxillary** and **vomerine teeth**.—These were examined in the skeleton. Note again their position, arrangement, prominence, etc.
- b.* The **posterior nares**.—How many are there? What is their position with regard to the anterior



nares? To the vomerine teeth? What is their shape? Pass a bristle through from the external openings.

Look back, near the union of the two jaws, and find

- c. **The openings of the Eustachian tubes.**—What is their exact position? How do they compare in size with the posterior nares? Pass a bristle into one of the openings, then remove the tympanic membrane of the same side. Into what cavity does the Eustachian tube lead?

Make a sketch of the roof of the mouth. Examine the floor of the mouth.

Do you find that any part of the floor is firmer than the rest? If so, where is this part, and to what is the firmness due?

- d. **The tongue.**—Does it entirely cover the floor of the mouth? What is its shape? Size? Where is it attached? Can the tip of the tongue be thrust out of the mouth? What is the character of the upper surface of the tongue? Note the **papillæ** covering this surface. Compare the lower with the upper surface. What reasons can you give for the shape of the posterior end?

Back of the tongue find an opening,

- e. **The glottis.**—What is its shape? What is the nature of its margin? What is the arrangement of the mucous membrane on each side of the glottis?

Pass a stiff guarded bristle back of the glottis, and note that it passes into another opening, which leads into the **œsophagus**.

In some frogs (males) the opening of a **vocal sac** may be found on each side between the edge of the tongue and the lower jaw.

II. *The alimentary canal.*

Lay the frog on its back and dissect away all of the muscles, bones, etc., covering the ventral side of the body from the pubis to the jaw, being careful not to injure the underlying parts. Leave the glottis and trachea in position. Note the manner in which the various organs—heart, liver, lungs, intestine, etc.—are closely packed. Insert a fine-pointed blowpipe—*e. g.*, a glass tube drawn out to a tapering end with rounded edge—into the glottis and inflate the lungs. This will define their position. Do likewise to the bladder by inserting the end of the blowpipe into the opening of the cloaca. Note the heart, almost surrounded by the lobes of the liver. Remove the heart, and at present pay attention only to the digestive organs.

- a. The **liver**. — What is its position? Shape? Color? Of how many lobes does it consist? What difference in their shape and size? What is its position relative to the heart? To the lungs? Do you find a partition or **diaphragm** completely separating the cavity in which the heart and lungs lie from that in which the liver and other digestive organs are found? Turn the liver over towards the left (the frog's right) side, and note the **oesophagus** leading into the **stomach**.
- b. The **oesophagus**. — In what direction does it run? What is its shape? Color? Diameter? What is its relation to the heart? To the lungs?

To the liver? How is the œsophagus held in place? Carefully pinch the œsophagus with the fine forceps. What is the nature of the wall? How do you distinguish the boundary between the œsophagus and the stomach? With the scissors make on the ventral side of the œsophagus a transverse slit, half severing it from the stomach, then from this slit make a longitudinal cut extending towards the mouth, thus laying open the œsophagus. Spread back the two flaps, remove the contents, if any, and examine the wall of the œsophagus. Do you find the lining membrane or **mucous membrane** arranged at all differently from the outer wall?

- c. The **stomach**.—On which side of the body does it lie? What is its shape? What is its relation to the liver? Note the membrane or **mesentery** which holds the stomach in place. Lay open the stomach by a cut extending along its left margin. Does its wall differ in any way from that of the œsophagus? Notice particularly the arrangement of the lining or **mucous coat**. Does this differ at all in stomachs which are distended with food and those which are empty?

Leading from the posterior end of the stomach find the **intestine**, consisting of a coiled portion, the **small intestine**, and a straight portion, the **large intestine**.

- d. The **small intestine**.—What is its position? Shape? Color? How is it arranged? Are its various coils connected? If so, by what means? How does it compare in diameter with the œsophagus? With the stomach? Is the wall of the

small intestine thin and flexible or thick and rigid? What is its length? The upper, nearly straight portion of the small intestine is called the **duodenum**; the lower, coiled portion is the **ilium**. Split open the small intestine, and compare its inner wall with that of the stomach. Note the shaggy appearance of the lining of the duodenum. Examine the transverse folds or **intestinal valves** in the ilium. Look for the valve, the **pylorus**, between the stomach and the duodenum.

*e.* The **large intestine**.—What is its position in the body? Shape? At what point on its surface does the small intestine unite with it? How is it situated with regard to the urinary bladder? Split the pubic bone apart, remove the two hind legs, and trace the large intestine down to the point where it unites with the **cloaca**. Examine the inside of the large intestine, and note the **valves** at the point of entrance of the small intestine.

*f.* The **cloaca**.—What is its position? Length? Structure of its walls?

Find, lying partly in the loop between the stomach and the duodenum,

*g.* The **pancreas**.—What is its relation to the stomach? To the liver? What is its texture? Shape? Color?

Spread apart the lobes of the liver and find a small yellow sac,

*h.* The **gall-bladder**.—Between which lobes does it lie? What is its position with regard to the heart? Look for the ducts, the **cystic ducts**, entering the gall-bladder from the liver.

Endeavor to trace the course of the **common bile duct**, which leads from the gall-bladder to the duodenum. This may be done by squeezing the gall-bladder, thus forcing some of its contents into the duct. What is the relation of the pancreas to the common bile-duct? Examine the inside of the duodenum for the opening of the gall-bladder.

Lying in the middle line, and to one side of the large intestine, find a small red body,

- i. The **spleen**.—What is its shape? Size? To what is it attached?

Cut the œsophagus across, as close as possible to the mouth, and carefully remove all of the digestive tract from the abdominal cavity by cutting the mesentery, which holds the parts in place. Uncoil the intestine in the same manner. Leave the large intestine attached to the cloaca. Do not disturb the other organs. Straighten the whole alimentary canal and measure its length. How many times longer than the body is it? How far from the anterior end do the digestive glands—liver and pancreas—pour their secretions into the canal? Note the pigmented membrane or **peritoneum** which lines the body-cavity or **pleuro-peritoneal cavity**. Note also that it is this membrane which forms a sling in which the stomach and other parts of the digestive system are held. By what is this sling supported?

Make a diagram of the entire alimentary canal.

#### *D.—The Respiratory System.*

Passing downward from the glottis find

- a. The **larynx**.—What is its position with regard to

the glottis? To the lungs? What is the structure of its walls?

Cut away the anterior wall of the larynx and note, leading to each lung,

b. The **bronchus**.—What is the shape of the bronchus? Length? Structure?

c. The **lungs**.—What position do the lungs occupy with regard to the larynx? The heart? The liver? How are they held in place? What is the shape of the lung when collapsed? When distended? How much larger than the former is the latter? What is the texture of the wall of the lung? On the distended lung look for the **pulmonary artery**, running the entire length of the organ. Distend one of the lungs, then remove it by cutting through the bronchus, and with a pair of fine scissors inserted into the bronchial opening slit the lung along one side to the end, and spread out the organ under water. What is the structure of the lung? Note the network formed by muscles, connective tissue, and blood-vessels.

d. The **vocal cords**.—Open the glottis and look for two narrow bands of tissue stretched vertically and running from before backward.

Make a diagram showing the arrangement of the respiratory organs. Draw a collapsed and a distended lung to the same scale. Make a drawing showing a cross-section of a distended lung; another drawing showing the network on the interior.

e. The **vocal sacs**.—Select a male frog and examine the vocal sacs. What is their position? Shape?

To what extent may they be inflated? Are they directly connected with the lungs?

*E.—The Urino-genital System.*

After the digestive organs have been removed, there will be found in both sexes two elongated, dark-red bodies lying on each side of the vertebral column. These are

- a. The **kidneys**.—What is their position with regard to the large intestine? To the spleen? What is their shape? How long are they? What is their relation to the peritoneum?

On the surface of each kidney find a yellowish band,

- b. The **adrenal body**.—What is its shape? How much of the length of the kidney does it cover? Of the width? How intimately is it connected to the kidney? Draw a kidney with its adrenal body.

Leading from the kidney find a white tube,

- c. The **ureter**.—From what part of the kidney does it arise? Is it straight or coiled? What is its length? Into what does it lead? Open the cloaca and endeavor to find the opening of the ureter. In the male this duct carries the secretion of the testes and is, hence, a **urinogenital** duct. In such a specimen examine the lower end of the duct for a dilated glandular portion, the **vesicula seminalis**.

- d. The **bladder**.—What is its position? What is its relation to the large intestine? Shape? Size? Texture of its wall? Is it directly connected with the ureters? How is it connected with the clo-

aca? How held in place? Make a sketch of the bladder.

In male specimens look near the kidneys for two yellowish bodies,

- e. The **testes**.—How are they situated with regard to the kidneys? What is their shape? Size? Color? How are they held in place? Look for the ducts or **vasa efferentia**, which run from the testes to the kidneys. At what point do these ducts leave the testes? Where do the ducts enter the kidneys? How many such ducts has each testis?

On the anterior end of each testis find a series of fringe-like lobes forming

- f. The **fat bodies**.—What is their exact position? Shape? Color? How many lobes has each? Make a sketch of a testis and the fat bodies.

In female specimens look, in quite the same position as the testes occupy in the male, for dark-gray or black bodies,

- g. The **ovaries**.—Compare them in shape, size, color, structure, etc., with the testes. When the ovaries contain eggs which are approaching maturity, the latter look like small shot, which distend the ovaries until they at times fill a very large part of the abdominal cavity. What is the shape of the eggs? Color?

In the neighborhood of these glands look for coiled white tubes,

- h. The **oviducts**.—How do they compare in size with the ureters? Carefully remove one of the oviducts, beginning at the posterior end, and note



which direction does it extend? Examine its surface with a lens. Do you find blood-vessels in its wall? How do you distinguish the aortic arches from the surrounding parts? In what direction do the arches run? Make a drawing of the heart, showing all of the parts thus far examined.

Cautiously bend the heart backward by raising its tip. On the dorsal side of the heart find the **sinus venosus**. What is its shape? How is it attached to the heart? How much of the latter does it cover? What is the texture of its wall? Find three veins, the **venæ cavæ**, one posterior and two anterior, leading into the sinus. At what points do they enter? Dissect away the ventral wall of the sinus and look for the opening, the **sinu-auricular aperture**, by which it communicates with the right auricle. What is the position of the aperture? Shape? Note the lip-like folds or **valves** which guard the opening.

## II. *The veins.*

- a.* The **anterior venæ cavæ**.—On either the right or left side trace the caval vein of that side by carefully dissecting away the muscles and membranes. It will be seen to be formed by the union of three veins—one, the **subclavian**, coming from the direction of the fore-limb; another, the **external jugular**, from the outer edge of the hyoid bone; and the third, the **innominate**, coming in between the other two. Which of these veins is the largest? Trace forward the two branches of the external jugular vein. One branch, the **lingual vein**, comes from the tongue and the floor of the mouth; the other

branch, the **inferior maxillary vein**, comes from the margin of the lower jaw. What is the course of each vein? Find the two branches which unite to form the subclavian vein; one, the **brachial vein**, comes from the fore limb, and the other, the **musculo-cutaneous vein**, comes from the skin and muscles of the side and back of the body. The branches of the innominate vein are the **internal jugular**, which appears immediately behind the angle of the jaw, and the **subscapular vein**, lying among the muscles of the shoulder. From what parts of the body does the blood come which enters the sinus venosus through the anterior venæ cavæ?

Make a diagram of the veins which communicate with the anterior venæ cavæ.

6. The **posterior vena cava**.—Displace the alimentary canal by cutting across the œsophagus and turning the entire system downward over the pubis, cutting the mesentery wherever necessary. In which direction does this vein run? How does it compare in diameter with the anterior venæ cavæ? Trace the vein towards the posterior end of the body and find coming from the liver the **hepatic veins**. How many are there? At what point do they enter the vena cava? Farther back find the **renal veins**, entering from the kidneys along with the **genital veins** from the ovaries or testes, as the case may be. How many renal veins do you find? How many genital? Which are the larger?

Make a diagram of the posterior vena cava and the connecting veins.

- c. The **pulmonary veins**.—Turn the heart forward and find coming from each lung a small, dark-colored vein. What is their position with regard to the sinus venosus? Which cavity of the heart do these veins enter? Do they unite before entering the heart?

Make a drawing showing the relation of the pulmonary veins to the heart.

- d. The **anterior abdominal vein**.—Examine the median ventral line of the body. What is the course of this vein? Note its branches, the **epigastric veins**. How are they arranged? How are they situated with regard to the abdominal muscles? Trace the anterior abdominal vein back to the pubis, and note that it is formed by the union of two short veins, the **pelvic veins**. Separate the muscles of one of the thighs and find the two veins, **femoral** and **sciatic**, which unite to form the pelvic vein. The femoral vein may be distinguished by its larger size. Does this vein communicate with the sciatic? Trace the femoral vein to the space back of the knee-joint, and note that this vein is a continuation of another, the **posterior tibial vein**, which arises from branches covering the upper surface of the foot. At the posterior end of the anterior abdominal vein find the **vesical vein**, coming from the bladder. Look also for the **parietal veins**, which come from the ventral side of the body-wall. On the truncus arteriosus find another branch, the **cardiac vein**. At the forward end of the anterior abdominal vein find its branches, which run to the liver. Does this vein communicate directly with the heart?

Make a diagram showing the vein and its branches.

- e. The **hepatic portal vein**.—Look for this vein near the point where the anterior abdominal vein sends its branches to the liver. Endeavor to find the branches—**gastric vein**, **intestinal vein**, and sometimes **splenic vein**—by whose union this vein is formed.

Make a diagram of the hepatic portal vein and its branches.

- f. The **renal portal vein**.—This vein is found entering the outer side of the kidney. Trace it backward to its branches, the **sciatic vein** from the thigh and the **dorso-lumbar vein** from the back, and, in female, the **oviducal veins** from the ovary.

Make a diagram of the renal portal vein and its branches.

### III. *The arteries.*

Take a fresh specimen and prepare it as directed for the study of the veins. The arteries may be distinguished as being of lighter color and having thicker walls than the veins. Distend the cesophagus by thrusting into it a slender pencil, a glass rod, or a roll of paper.

- a. The **aortic arches**.—Trace forward the right and left branches of the truncus arteriosus, cleaning away the muscles and connective tissue, and notice that each branch subdivides into three sets of tubes which form the aortic arches. These are the **carotid arch**, which is the anterior of the three; the **systemic arch**, which lies between the other two, and the **pulmo-cutaneous arch**. How

far from the heart do the arteries arise? How far apart? Trace the anterior artery, the **common carotid**, to the point where it subdivides into the **external carotid** or **lingual artery** and the **internal carotid artery**. What course does each take? What is the position of the lingual artery with regard to the external jugular vein? Of the internal carotid artery with reference to the internal jugular vein? Near the base of the lingual artery find a small swelling, the **carotid gland**.

Make a diagram of the carotid or aortic arch and its branches.

Study next the systemic arch. Trace either half, dorsally and posteriorly, to the anterior end of the kidney, where it unites with the corresponding artery from the other side and forms the **dorsal aorta**. Still farther back it divides into the **iliac arteries**. How long is the aortic arch? How long is the dorsal aorta? At what point do the iliac arteries arise? In which direction do they run? Given off by the aortic arch find the **subclavian artery**. In which direction does it run? What is its relation to the subclavian vein? Find also the **vertebral artery**. What is its position? Note its branches. How are they arranged? How many are there? To what parts do they run? At or near the point where the dorsal aorta arises look for the stump of a branch, the **coeliaco-mesenteric artery**, which runs to the alimentary canal. Trace the branches of this blood-vessel to the various digestive organs. Farther back find the **urino-genital arteries**. From what part of the dorsal aorta do they arise? How many are there? Running to the large intestine

find the **inferior mesenteric artery**. Look also for the **splenic artery**. Trace one of the **iliac arteries** back to the **sciatic artery**, which eventually divides into **peroneal** and **tibial arteries**.

Make a diagram of the arrangement of the blood-vessels which communicate with the heart by means of the systemic arch.

Examine the pulmo-cutaneous arch, and trace its branches, the **cutaneous artery**, to the skin, and the **pulmonary artery**, to the surface of the lung. Draw this arch and its branches.

Compare the above arches as regards the extent of the distribution of their branches.

#### IV. *The internal anatomy of the heart.*

In frogs killed with chloroform it will be found that the heart is distended with blood. The heart of any of the specimens thus far examined may be used, or one may be especially prepared by killing a frog with chloroform, opening the body so as to expose the heart, and placing the entire specimen in alcohol. In a day or so remove the heart by cutting it away from the lungs and severing its attached blood-vessels at a sufficiently remote distance from the organ. Examine its external anatomy again so as to get the various regions of the organ clearly in mind. Select three small bristles of different colors, and gently thrust one of them into the carotid, another into the aortic, and the third into the pulmonary arch. Then fasten the heart down, dorsal side uppermost, in a dissecting-pan by thrusting small pins through the apex of the ven-

tricle and through some of the blood-vessels. With sharp, fine scissors carefully cut away enough of the ventral surface of the ventricle, truncus arteriosus, auricles, and aortic arches to expose their cavities. With a stream of water from a pipette wash the clotted blood out of the cavities. The study of certain points will be facilitated if the preparation be placed in a deep watch-glass containing fifty per cent. alcohol, and held near a window where the light may shine through the membranes. Examine the **ventricle**. What is the character of its wall? How thick is it? What is its color? How does it differ from the walls of the **auricles**? From that of the **truncus arteriosus**? Notice that the cavity of the ventricle is undivided. On the right of the preparation look for the **auriculo-ventricular aperture**. What is its shape? Into what does it open? Look for the two **auriculo-ventricular valves**. How are they connected with the walls of the ventricle? Between the two auricles find the **inter-auricular septum**. What is its structure? In which direction does it extend? What is its position with regard to the truncus arteriosus? To the auriculo-ventricular aperture? In the right auricle, near the septum, look for the opening of the sinus venosus, the **sinu-auricular aperture**. Near the top of the left auricle find close to the septum the **aperture of the pulmonary veins**. Examine the truncus arteriosus and note its connection with the ventricle. From what part of the latter does the truncus start? Note the **longitudinal valve** which divides the truncus into two parts.

At what points do the carotid, aortic, and pulmonary arches unite with the truncus?

Make a drawing illustrating the structure of the heart and the connecting blood-vessels.

V. *The lymph hearts.*

The study of the lymphatic system is too difficult for the beginner. The **posterior lymph hearts**, however, are easily found by dissecting away the skin on each side at the posterior end of the urostyle. The pulsations of these organs are sometimes visible through the skin in the living frog. It is best to look for them immediately after the frog's death, before they cease moving and partly collapse.

G.—*The Nervous System.*

Select a large specimen which has lain for two or three days in seventy-five per cent. alcohol, portions of the skull and vertebral column having been removed in order to permit the alcohol to penetrate to the enclosed organs. Clean away all of the dorsal muscles on each side of the vertebral column, from the sacrum to the base of the skull. Remove the roof of the skull by inserting the points of fine, strong scissors through the membrane covering the space between the base of the skull and the first vertebra, and making parallel incisions passing forward on each side from the occipital region to the nostrils. Be exceedingly careful not to run the points of the scissors into the brain. As rapidly as the skull is loosened behind, lift it up with forceps and turn it forward in order to see where to cut. Do



not injure the pigmented membrane covering the brain. When the roof of the skull has been removed, cut away in the same manner the tops of the neural arches and urostyle. Prepare another specimen by fastening it down on its back and removing all of the viscera.

I. *General structure.*

- a. Compare the two specimens and note that the nervous system consists of two portions, a **central portion**, enclosed in the **cerebro-spinal canal**, and a **peripheral portion**, consisting of nerves running to various parts of the body. Notice also that the central nervous system is divisible into two regions, an anterior portion, the **encephalon** or **brain**, lying in the skull, and a posterior portion, the **myelon** or **spinal cord**, lying in the neural canal. How do these two regions compare in length? In diameter? Are they distinctly separated from one another? How do you distinguish the one from the other?
- b. The **membranes**.—Examine the pigmented membrane, the **pia mater**, which covers these parts of the nervous system. What is its color? Is it evenly colored? If not, where are there noticeable collections of pigment cells? Does the pia mater cover all parts lying within the cerebro-spinal canal? Note the large blood-vessel running along the middle of the dorsal surface of the pia mater. Examine the membrane with a lens, and note the numerous blood-vessels. Divide the pia mater immediately behind the posterior lobes of the brain, and examine that part of it which, forming a **choroid plexus**, covers a triangular cav-

ity, viz., the fourth cerebral ventricle, immediately below the pia. Is the choroid plexus thicker than other parts of the membrane? Is it more vascular? Lining the wall of the neural canal look for another membrane, the **dura mater**. Is it colored like the pia? Is it as thin and vascular as the latter?

## II. *The central nervous system.*

a. **The brain.**—Examine its dorsal surface. What is its general shape? Is it bilaterally symmetrical? Is its surface smooth, or marked with ridges and furrows, *i. e.*, convoluted? What is its position with reference to the eyes? Lying between the latter organs find on the brain two elongated masses, the **cerebral hemispheres**, at the anterior end of each of which is an **olfactory lobe**, from which proceeds forward a nerve, the **olfactory nerve**. What is the shape of the cerebral hemispheres? Why are they called “hemispheres”? How long are they? How wide? Are they connected? If so, how? How much of the brain do they form? Are the olfactory lobes sharply separated from the cerebral hemispheres? Are the lobes connected with each other? What is their shape? How do they compare in size with the hemispheres? Immediately behind the hemispheres find a diamond-shaped area, the **thalamencephalon**. Note that the pia mater here forms another choroid plexus. Near the middle of the roof of the thalamencephalon find the remains of the **stalk of the pineal gland**, the gland probably having been torn away when the skull was removed. Cut away the choroid plexus and

beneath it find a cavity, the **third ventricle**, in the thalamencephalon. Notice the thickened sides of the thalamencephalon which form the **optic thalami**. Posterior to the thalamencephalon find the two **optic lobes**. How do they compare in size with the cerebral hemispheres? With the olfactory lobes? What is their shape? Behind the optic lobes find a transverse band, the **cerebellum**. What is its shape? Back of the cerebellum comes the **medulla oblongata**, which gradually tapers into the spinal cord. What is the shape of the medulla? Can you distinguish a dividing line between the medulla and the cord? In the medulla find a cavity, the **fourth ventricle**. Is the ventricle continued under the cerebellum? Does it extend back into the spinal cord? Draw the dorsal surface of the brain.

With a sharp scalpel or scissors cut off horizontally the upper surface of the brain, thus exposing the cavities or **ventricles**. Notice that the brain is *hollow*, with its walls variously bent and folded. Notice also that some of the cavities lie in the *median* or *axial line*, and that the others are *paired* lateral outgrowths of the former. Do the walls of the parts of the brain vary in thickness in different regions? Begin at the fourth ventricle and trace forward the system of cavities. What parts of the brain form the walls of this ventricle? Find the duct, the *iter a tertio ad quartum ventriculum* or the **aqueduct of Sylvius**, which leads from the fourth ventricle forward to the third ventricle. What is the relation of this duct to the optic lobes? Are the latter hollow? If so, what is the shape of the cavities?

Do the cavities communicate with the duct? What is the shape of the third ventricle? Note the **lateral ventricles**, one in each of the cerebral lobes. What is their shape? Do they extend forward into the olfactory lobes? Look for an opening, the **foramen of Monro**, leading from the third into each of the lateral ventricles. What is its position?

Make a diagram showing the cavities of the brain.

Make an incision separating the olfactory nerves from the lobes; gently lift up the anterior end of the brain, and bend the latter backward until it rests upon the spinal cord. In doing so cut each of the nerves passing out through the cranial walls. The ventral surface of the brain will then be exposed. Note the pia mater with its blood-vessels. Remove the pia. Can you trace the **ventral roots of the olfactory nerves** running back upon the olfactory and cerebral lobes? Are the cerebral lobes entirely separated at any point on their ventral surface? Between the bases of the latter lobes find a rounded surface, the **lamina terminalis**. Extending around the posterior portion of the lamina find the **roots of the optic nerves**, connected in the middle line by the **optic chiasma**. Running dorsally from the chiasma on each side is an **optic tract**. What is the appearance of the optic lobes as seen from below? What is their position with regard to the optic nerves, chiasma, and tracts? Immediately behind the chiasma find a lobed body, the **tuber cinereum**. What is its shape? What is its relation to the thalamencephalon? Directly back of the tuber find the stalk, the **infundibulum**, of the

**pituitary body or hypophysis.** What is the shape of the latter? Color? Size as compared with the tuber?

Draw the ventral aspect of the brain.

Examine another brain which has been hardened and removed from the skull, and note the various parts as seen from the side. Draw the lateral view of such a preparation. Make vertical sections, both transversely and longitudinally through a brain and study the sections in order. Draw.

- b. **The spinal cord.**—Study carefully the shape of the spinal cord as seen from above. Notice the **enlargements, brachial and lumbar**, at the points where the nerves for the limbs originate. Note that the posterior end of the cord tapers into the **filum terminale**, which extends into the canal of the urostyle. Does the cord fill the neural canal? Note this particularly with reference to the posterior portion of the neural canal where the end of the cord, together with the **roots** of some of the nerves which run to the hinder parts of the body, forms the **cauda equina**. Look for the **dorsal fissure**, which extends along the cord. How far can you trace it? Examine a cord which has been removed from the spinal canal. Can you find a ventral fissure? Do these correspond to parts seen on the brain? Examine a transverse section of a cord and find the **central canal**. To what does this correspond in the brain? Note also the roots of the **spinal nerves**.

Make the drawings necessary to show the structure of the spinal cord.

### III. *The peripheral nervous system.*

With the experience which he has now had in dissecting muscles and in tracing blood-vessels, the student ought to be able to trace the following nerves, and to distinguish their more striking features; consequently only the names, origin, and termination of the parts of the peripheral nervous system will be given.

#### a. *The spinal nerves.*

Examine a spinal cord and note the connection of each of the spinal nerves to the spinal cord by means of an **anterior** or **ventral** and a **posterior** or **dorsal root**. In which direction do these roots run? Is there any difference in this respect between the roots of the anterior and those of the posterior nerves? Trace the roots to the intervertebral foramina. Do the roots unite inside the spinal canal? Turn the frog over on its back, and note the nerves lying as white cords on each side of the vertebral column. At their point of issue from the spinal column they are covered by a yellowish-white deposit of lime secreted by the **periganglionic glands**. This may be neatly removed by gently teasing with the point of a fine, sharp scalpel the pigmented membrane which covers the limy patches, then with a pipette carefully placing on each patch a drop of dilute nitric or hydrochloric acid. The acid dissolves the lime without injuring the surrounding tissues.

Under the patch may be found the point of union of the two roots. Towards the cord from this point look on the posterior root for the **gan-**

**g**lion. How do you distinguish it? What is its shape? Size? Color? Immediately after the two roots unite, the nerve divides again into two branches, one of which runs dorsally, the other ventrally. How do the two branches compare in size? The dorsal branches run mainly to the muscles and skin of the back.

Trace, in order, the following ventral branches of the spinal nerves.

These nerves receive their number from the number of that vertebra back of which they leave the neural canal.

1. The **first spinal nerve** or **hypoglossal** runs forward from behind the first vertebra to the tongue, passing in its course under the mylohyoid muscle and into the substance of the geniohyoid.
2. The **second and third spinal nerves** unite to form the **brachial nerve**. This gives off a branch to the shoulder muscles and then continues down the arm to form the **radial and ulnar nerves**.
3. The **fourth, fifth, and sixth spinal nerves** run to the body-wall and to the skin.
4. The **seventh, eighth, and ninth spinal nerves** unite to form the **sciatic plexus**, from which arises the **sciatic nerve**, which continues down the hind limb and divides into the **tibial and peroneal nerves**.
5. The **tenth spinal nerve** or **coccygeal** emerges from a foramen in the urostyle, and sends branches to the cloaca, bladder, and surrounding parts.

Compare all of these nerves and note the striking dif-

ferences in size, color, direction, branches, etc. Make a diagram of the spinal nerves as seen from the ventral side.

*b. The Sympathetic System.*

This is best seen in a specimen from which the internal organs have been removed from one half of the body. Push the remaining organs to one side to see the nerves which supply them. The principal part of the sympathetic system consists of a row of nerves and ganglia lying on each side of the ventral median line of the spinal column. From the ganglia and nerves arise branches which run to the lungs, heart, principal blood-vessels, stomach, intestine, liver, kidneys, genital glands, etc.; to the spinal and cranial nerves; and between the various parts of the sympathetic system.

How do the sympathetic compare in size with the spinal nerves? How many sympathetic ganglia do you find? What noticeable differences between these and the spinal ganglia can you detect?

Make a diagram of the sympathetic nervous system.

*c. The cranial nerves.*

To see these well, two specimens should be prepared, one to show a ventral view of the inside of the skull, the other to show the ventral surface of the brain. The second may be prepared by removing one half of the floor of the mouth, the mucous membrane of the roof of the mouth, and the floor of the cranium and of the auditory capsules. Especial care must be exercised in order not to tear away the nerves from the parts to which they are connected.



The cranial nerves are numbered in the order of their origin from the anterior towards the posterior part of the brain :

1. The first, or **olfactory nerve**, runs from the olfactory lobe to the membrane lining the nasal cavity.
2. The second, or **optic nerve**, arises below the optic lobe, and passes through the optic chiasma to the eye on the opposite side.
3. The third, or **motor oculi**, originates on the ventral surface of the brain near the pituitary body, and supplies some of the eye muscles.
4. The fourth, or **pathetic**, arises on the dorsal surface of the brain in front of the cerebellum, leaves the cranium in front of the optic nerve, and runs to one of the eye muscles.
5. The fifth, or **trigeminal**, runs forward from the anterior part of the medulla to leave the cranium immediately in front of the auditory capsule. Just within the place of exit look for the **Gasserian ganglion**. What is its exact position? Compare it with the spinal and with the sympathetic ganglia. On the outer side of the ganglion the nerve divides into two branches: the first or **ophthalmic nerve** runs to the skin of the front part of the head and to the nasal cavity; the second or **maxillo-mandibularis** divides into two branches, the **maxillary nerve**, which runs to the skin of the upper jaw and to the lower eyelid, and the **mandibular nerve**, which supplies parts of the lower jaw.
6. The sixth, or **abducens**, originates near the median ventral line of the medulla, behind the

pituitary body, runs very near the Gasserian ganglion, and supplies some of the eye muscles.

7. The seventh, or **facial**, arises immediately behind the fifth, runs forward to the Gasserian ganglion, and after leaving the cranium divides into two principal branches, one of which, the **palatine nerve**, supplies the roof of the mouth, while the other or **hyomandibular nerve** sends branches to the mandible, hyoid bone, and ear.
8. The eighth, or **auditory nerve**, arises close to the seventh and passes to the internal ear.
9. The ninth, or **glossopharyngeal nerve**, originates immediately behind the auditory nerve. Of its two main branches, one unites with the facial nerve and the other runs to the tongue and pharynx and the neighboring muscles.
10. The tenth, the **pneumogastric** or **vagus nerve**, arises with the ninth and sends branches to the larynx, the lungs, the heart, and the stomach. Look for a ganglion on this nerve.

Compare the cranial with the spinal nerves, noting any resemblances and differences that you may discover. Do the cranial nerves originate from anterior and posterior roots? To what region of body are these nerves mainly confined?

Make diagrams showing the origin and course of the cranial nerves.

Let the student compare the nervous system of a frog with that of man as illustrated by a manikin or chart.

*H.—The Eye and the Ear.*

- a. **The eye.**—Examine the eye of a living frog, and make out the transparent outer portion or **cornea**, below which lies a pigmented ring, the **iris**, in the centre of which is a black opening, the **pupil**. What is the color of the iris? How much of the exposed portion of the eyeball does it form? What is the shape of the pupil? Diameter? Watch the eye carefully to see if the pupil changes in size. Kill the frog with chloroform, and remove the eyes by cutting the eyelids and muscles. Note how the lids are connected to the eyeball by means of the **conjunctiva**. Examine again the shape of the eye, and find the point of entrance of the **optic nerve**. Notice the white **sclerotic membrane** which forms the outer coat of the eye. Place the eyes in a watch-glass of water, and with sharp scissors divide one by a cut passing through the centre of the pupil and slightly to one side of the optic nerve; the other by an incision at right angles to the line passing through the pupil and the nerve. With a lens study the sections first made and notice the black **choroid membrane**, which lines the cavity or **vitreous chamber**, in which lies the **vitreous humor**. What is the relation of this membrane to the sclerotic? To the iris? What is the appearance of the vitreous humor? Remove it and examine its shape. Anterior to the vitreous humor find the **crystalline lens**. What is its shape? Size as compared with the vitreous humor? In front of the lens is the **anterior chamber** of the eye containing the **aqueous humor**. Examine the sections of the other eye,

and in that section which contains the outer half study the pupil. Why is the pupil black? From the inner section remove the vitreous humor, and back of it find a delicate membrane, the **retina**, covering the choroid. Look also for the point of entrance of the optic nerve, the **blind spot**.

Make a drawing showing the eye in antero-posterior section ; another to show the layers of membrane in the posterior half of the eye.

- b. The **ear**.—The parts of the internal ear may best be dissected in preserved specimens which have been macerated in a mixture of sixty per cent. alcohol, to which a few drops of nitric acid have been added. Leave the specimen in the mixture until the muscles and bones assume a translucent appearance, then, with forceps, pick away the skin and muscles surrounding the auditory capsule, tear off the top of the latter, which now has the appearance and consistence of cartilage, and within will be found the **membranous labyrinth**, which appears as a small white nodule, to which are attached grayish pigmented parts. Float the labyrinth into water in a watch-glass and examine it with a lens. The white portion, or **vestibule**, consists of two parts separated by a constriction, the lower part being the **sacculus** and the upper the **utricle**. From the upper arise the three **semicircular canals**, each of which bears at its base an enlargement, the **ampulla**. Endeavor to trace the connections of the **auditory nerve**.

Make a drawing showing the relation of these parts.

*Microscopic Anatomy.*

From the freshly killed frog and in the manner described the student will make and study preparations of the various parts named hereafter. These are such as may readily be made on a glass slide without elaborate manipulation. As only a few structures can be studied in this way, he should also have for examination a set of permanent preparations of various organs which have been hardened, stained, sectioned on a microtome, and mounted in Canada balsam. This material will be provided by the instructor, as the student has not yet had sufficient training in histological technique to enable him to prepare satisfactory specimens. Before staining a preparation always examine it in the fresh state in a drop of water or of normal salt solution. Make drawings and full notes of every specimen studied.

*a. The skin.*

Remove pieces of skin from the various places on the dorsal and ventral surfaces of the body and from the limbs, mount in a drop of water, and look for the **pigment cells** and the openings of the **cutaneous glands**.

*b. The blood.*—See page 23.*c. Connective tissue.*

1. **Fibrous tissue.**—Tease a bit of the *tendo Achillis* in water. Note the arrangement and structure of the **white, non - elastic fibres**. Do they branch? Apply a drop of dilute acetic acid. Look in this tendon and in those from other parts of the body for **yellow, elastic**

**fibres.** Compare with the white. Look also for **connective-tissue corpuscles.**

2. **Areolar tissue.**—Without stretching it, lay in a drop of water on a slide a portion of the thin, web-like tissue which connects many of the adjacent muscles, or, in places, attaches the skin to the underlying parts. What kinds of connective tissue can you find? Stain with magenta. Examine another specimen which has been treated with a one-half per cent. solution of silver nitrate for three to five minutes, then washed with distilled water and left exposed to the sun until brown.

*d. Hyaline cartilage.*

Dissect out the xiphisternum, remove its covering membrane, the **perichondrium**, cut off a piece of the cartilage, and mount in a drop of salt solution. Note the arrangement of the **cartilage corpuscles** embedded singly or in groups in the **matrix**. Look for **nuclei**, particularly such as show by their elongation, etc., that they are in the process of division. Wash the specimen in water, stain with an aqueous solution of hæmatoxylin, and examine in glycerine.

*e. Adipose tissue.*

Cut off a portion of a fat body, tease it in a drop of salt solution, and study the structure of the tissue, which consists mainly of connective tissue and **fat cells**. Treat with chloroform.

*f. Muscular tissue.*

1. **Unstriated muscle.**—Remove a portion of the outer layer of the intestine and tease it in a drop of salt solution. Look for long, spindle-shape

cells. Apply acetic acid. Treat another preparation with an aqueous solution of hæmatoxylin.

2. **Striated muscle.**—Remove a portion of the gastrocnemius and examine as above. Notice that the muscle consists of fibres. In the latter look for the **striations**, the **nuclei**, and the membrane or **sarcolemma**. Treat one preparation with acetic acid, another with magenta.

*g. Nervous tissue.*

1. **Nerve fibres.**—Remove a piece of the sciatic or other large nerve and carefully tease it in a drop of salt solution. Note the **medullated nerve fibres** held together by a membrane, the **perineurium**. Examine a single fibre and endeavor to make out the surrounding membrane, called the **primitive sheath** or **sheath of Schwann**. Within this is the **medullary sheath**. The former is best seen in fibres which have been torn. In such fibres look also for the **axis cylinder**, projecting beyond the medullary sheath. Treat a preparation with chloroform, which will partially dissolve the fatty substance forming the medullary sheath, and thus make the primitive sheath and the axis cylinder more plainly apparent.
2. **Nerve cells.**—Remove a spinal ganglion and tease it in eosin. Examine the preparation for large spherical cells, each with a conspicuous nucleus. Examine in the same manner the cells from one of the sympathetic ganglia.

*h. The liver.*

Cut off a small piece of a fresh liver, tease in a drop of salt solution, and look for the **hepatic**

**cells.** Treat the preparation with acetic acid. Treat another preparation with iodine; the deep red color assumed by the contents of certain cells indicates the presence of **glycogen**.

*i. The testis.*

In a drop of water finely divide a portion of the testis of a recently killed frog, and look for actively moving **spermatozoa**. Apply magenta. To another preparation apply dilute iodine.

PHYSIOLOGY

*A. Locomotion.*

Does the frog ever walk? Run? Can it walk backward? Leap backward? Walk sidewise? Swim backward? In swimming, what use is made of the fore limbs? Can it float?

*B. Nutrition.*

*a. Feeding.*—Place a frog in a low glass jar, beaker, or tumbler, along with a living fly. Watch the frog, to see how it catches the fly, noticing particularly the action of the tongue. If it be otherwise impossible to get the fly within reach of the frog, kill the former by pinching its head, run a fine thread through its body by means of a needle, then lower the fly, dangling at the end of the thread, into the jar. If flies cannot be obtained, use a small piece of fresh meat. Can you give any reasons for the structural arrangement of the frog's tongue?

*b. Breathing.*—Watch the nostrils of a frog. Can you detect any motions? Watch the throat and the sides of the abdomen. Do they move? If so, do they move at the same time? While holding your hands on your sides, draw in a breath. Do



your ribs move? Can you inspire deeply without moving the ribs? Has the frog ribs, or anything corresponding to them? If so, are these parts movable? Can they assist in inspiration? Does the frog breathe as you do? Why can a frog stay under water for several days without coming to the surface to breathe? Why do frogs die when their skin becomes dry?

*c. Circulation.*

1. *The beat of the heart.*—Chloroform a frog and expose its heart. Note the beat of the heart, consisting of a contraction or **systole**, followed by an expansion or **diastole**. How rapid are the pulsations? How do the ventricle and the auricles behave? Do any of the parts change color during the beat? Shape? Size? With the finger lightly touch the various parts of the heart while it is beating, and note the variations in tension of the walls. Remove a heart from the body by cutting the blood-vessels, and immerse it in a salt solution in a watch-glass. Does the heart still beat, though it contain no blood? If so, how long does its activity last?
2. *The circulation through the web.*—Provide a piece of thin board—*e. g.*, such as is used for the backs of pictures—about four or five inches long and two or three inches wide. In the middle of one end bore a hole or cut a notch, over which the web of the frog's foot may be stretched. Render the frog insensible with chloroform, but do not kill it. Lay it on the board, ventral side downward, and fasten it with a tape passed firmly, but not tightly, around its body a few

times. Make a slip noose in the ends of three or four threads, tighten the nooses around the tips of the toes, and by fastening the threads at different points on the board, lightly spread the web over the hole. Keep the frog under the influence of chloroform, and keep its body moist by spreading over it a damp cloth or a layer of wet, absorbent cotton. Examine the web with a low power, noting the pigment cells and the blood-vessels. Put a drop of water on a part of the web, lay on it a bit of cover-glass which will fit between the toes, and examine the preparation with a high power. Study the flow of blood in the various vessels, and note the behavior of both red and colorless corpuscles.

### *C. Development.*

In the early spring, when the ice is leaving the ponds and streams, frogs' and toads' eggs may be found in abundance as slimy masses fastened to reeds and water-plants, or floating on the surface. Each mass consists of a number of small, black, berry-like bodies, each of which is embedded in a gelatinous matrix. One of these masses should be transferred to the laboratory, placed in a large jar of water, which should frequently be changed, and the eggs examined from day to day. The various stages of segmentation may be studied, and the development of the tadpoles watched. As soon as the latter appear, they must be provided with a supply of water-plants to which they may attach themselves.

For comparison with the frog make a general examination of a fish or a bird.



**PART III**  
**THE BIOLOGY OF THE PLANT**



## THE BIOLOGY OF THE PLANT

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### **Green Felt (*Vaucheria* Sp.)**

*Material.*—Various species of *Vaucheria* grow as a coarse, dark-green, felted mat on the rocks and timbers in streams, and on the surface of the earth in the beds and flower-pots in greenhouses. In the latter situation they may be had all the year round. The terrestrial species may be kept growing indefinitely by transferring to the laboratory some of the earth with the plant and covering it with a bell-jar. The aquatic species should be put into a low aquarium with plenty of mud and flat stones, and be kept covered with water three or four inches deep. In both situations the plant needs plenty of sunlight. Alcoholic material may be prepared according to the directions given for *Spirogyra*.

Iodine, carmine, Schulze's solution, two per cent. salt solution, saucer, fine forceps, pipette, hand-lens, etc., will be used in the examination.

*Method of Examination.*—The structure of this plant necessitates the most careful handling, in order that good specimens may be obtained. A small portion of the felted mass should be placed in a small dish of water, and gently shaken until some of the plants separate from the mass and float in the water. With a pipette

transfer two or three of these to a slide, and cover with a glass supported by a piece of hair or a scrap of paper.

#### MORPHOLOGY

##### A.—*Vegetative Condition.*

- I. *Naked-eye characters.*—Examine the plant as it grows in the water or on the surface of the soil. Compare it with *Spirogyra* in mode of growth, color, length of filament, etc. Does it have organs of attachment, as roots? Does it have the slimy feel of *Spirogyra*? Do you ever find it forming a frothy scum on the surface of the water? Why? Do the tips of the filaments ever project above the surface of the water? Why? Do these tips point towards the sun or away from it? Do you find any difference in the direction of the tips of filaments exposed to strong sunlight and those exposed to less intense light? Why is it called "Green Felt"?
- II. *Microscopic characters.*—Examine first with a low, then with a high power.
  - a. *Shape.*—What is the shape? Is the filament simple or branched? If branched, do the branches originate at any particular points? What relation of position exists among the branches? Does it have roots? What is the shape of the end of the filament? In what respects does this plant differ in shape from *Spirogyra*?
  - b. *Size.*—How long is a filament? Is its diameter greater or less than that of the species of *Spirogyra* examined? Does it have the same diameter throughout its entire length?

c. *Color*.—What is it? Is it evenly or unevenly distributed? To what is it due? Is it darker or lighter than *Spirogyra*? Why?

d. *Structure*.—Is the filament composed of cells? How many? How are the cells situated with regard to one another? Compare with *Spirogyra*. Can you distinguish cell-wall, vacuole, nucleus, starch grains, pyrenoids, etc.? Do you find **chromatophores**, as **chlorophyll bodies**? What is their shape? Arrangement? How do they compare in size, color, arrangement, shape, etc., with those of *Spirogyra*? Look for chlorophyll bodies in the process of division. Plasmolyze the filament with two per cent. solution of salt or sugar in water, and examine the **primordial utricle**. Does the cell-wall show any protuberances, stratification, or openings? What is its color? Look for spherical, glistening **oil-drops** in old filaments. Is the surface of the filament of this plant as clean and free from other organisms as is *Spirogyra*? Does *Vaucheria* have the characteristic mucous coating of *Spirogyra*? What is there about *Vaucheria* which enables it to form a "felt"? Why cannot *Spirogyra* do the same?

Make drawings illustrating all of the structures examined.

*B.—Reproductive Condition.*

Can you identify the reproductive stage without making a microscopic examination? How? Find some plants bearing sexual organs, which may be distinguished as short outgrowths from the side of the filament.



I. *The sexual reproductive organs, or gametangia.*

On mature specimens look for the curved **antheridia** and the oval **oögonia**. On what part of the plant are they found? Does the same plant bear both organs? How many of each do you find on one filament? How are they situated with regard to each other? What differences do you find in shape, size, and color?

- a. The **antheridium** or **male organ**.—What is its shape? Compare its diameter with that of the filament. Note the **pedicel** or stalk—its shape and structure. What does it contain? How is it connected to the filament? Does its cavity communicate with that of the filament? With that of the antheridium proper? Look for **antherozoids** or sperm-cells in the antheridium. What is their shape? Color? Arrangement in the antheridium? Examine a number of specimens to find antheridia in various stages of development. If any are found, make sketches of them. Look for empty antheridia. In what respects do they differ from the other? Look for antheridia from which the antherozoids are escaping, and study the details of the process—how the antherozoids are set free, their motions, and general behavior after leaving the antheridium. If antherozoids are found, apply iodine in order to see their **cilia**. How many have they?

- b. The **oögonium** or **female organ**.—Compare with the antheridium in shape, size, color, and mode of attachment to the filament. Is its cavity continuous with that of the filament? Does it have a pedicel? Endeavor to trace the develop-

ment of the oögonium by examining specimens which show it in various stages of growth. Of how many cells is it composed? Compare with antheridium. Try to see the manner in which a mature oögonium opens by the top becoming gelatinous. Endeavor to see the antherozoids enter. Examine oögonia which have been fertilized. What changes have taken place in the color, shape, and arrangement of the contained protoplasm, or **oösphere**, and in the cell-wall? Is the oögonium a motile organ? Is the fertilized oögonium in actual contact with the antheridium? Compare with *Spirogyra*. What is the structure of the ripe oösphere, now called **oöspore**? Are there any organs of motion on it? Compare with the zygospore of *Spirogyra*.

Draw a filament showing the facts learned about the reproductive organs of *Vaucheria*.

## II. *The asexual reproductive bodies.*

- a. The **zoögonidia** or **zoöspores**.—Place some vigorous plants in water in a dark place overnight, then examine for zoögonidia early the next morning, or keep the plants in the dark until ready to make the examination. Put some of the plants into a porcelain dish, and with a hand-lens look for filaments having swollen ends. These filaments are ready to form zoögonidia. Mount some of these filaments without a cover-glass. How much of the filament is occupied by the zoögonidium? What is the shape of a zoögonidium? Color? How does it move? What are its organs of motion? Where are they situated? What appropriateness about the name "zoögonidium"?

“Zoöspore”? Stain with iodine, and look for nuclei. Treat a moving zoögonidium with two per cent. salt solution to plasmolyze it. Do you find a cell-wall apparent? Treat in like manner a zoögonidium which has come to rest, and compare it with the first.

Try to find filaments in which the formation of zoögonidia is going on, and study the process. Is the zoögonidium formed by the union of sexual cells? Is the protoplasm of the zoögonidium separated from that of the cavity of the filament? Where a mature zoögonidium is found, watch to see it escape from the filament.

Study a zoögonidium which has come to rest, in order to see the process of germination.

*General Questions.*—As regards *structure*, is *Vaucheria* more or less complicated than *Spirogyra*? As regards *reproduction*, which is the more complicated? What is the advantage in having zoögonidia?

What means of dispersal has *Vaucheria*? Of protection?

Draw a filament, showing the stages in the formation of a zoögonidium, a mature zoögonidium, and the germination of the same.

### **Stonewort (*Chara* Sp.)**

*Material.*—Species of *Chara* are to be found growing in tangled masses in the bottoms of ponds, ditches, and slow-flowing streams. A handful of the plants may be pulled out of the mud and placed in a deep aquarium jar having a layer of mud two or three inches deep in the bottom. The plants should have an abundance of water. Small snails feed upon them, and there will probably be a good many of these attached to the plants. All but a few of these snails should be removed, otherwise they will injure and possibly destroy the plants by eating the buds and young branches. The fruiting stages are to be found during the late summer and fall. Aquarium specimens may continue to fruit until late in the winter. It is well, however, to preserve the fruiting stages when found, rather than run the risk of not having them fresh when wanted. Plants may be preserved in alcohol, passing them through the various grades from fifty per cent. to ninety-five per cent. as usual; or, better, place them for a day in one per cent. solution of chromic acid, wash for ten to fifteen minutes in running water, then place successively in fifty per cent., seventy per cent., eighty per cent., and ninety-five per cent. alcohol for about twelve hours each.

The student will need dissecting-needles, hand-lens, compound microscope, glass or porcelain bowl, fine forceps, dilute hydrochloric acid, pith, razor, pipette, watch-glass, a pair of dividers, iodine, carmine, magenta, fifty

per cent. glycerine, metric scale, scalpel, and chromic acid.

*Method of Examination.*—First examine the plants as they grow in a mass in the pond or aquarium, then carefully remove a plant from the tangle without breaking any parts, and study its gross anatomy by floating it in a dish of water, if the plant be living, or in fifty per cent. alcohol if the specimen has been preserved.

#### MORPHOLOGY

##### *Naked-eye Characters.*

- a. *General appearance.*—Note the plant as it grows in the pond or aquarium. What is the color of the mass? Is the mass dense or loose? Lift a handful. Is it heavy or light? How does the plant feel to the touch? Compare with *Spirogyra* and *Vaucheria* in this respect. Can you suggest any reasons why the plant should be named “Stonewort”? Does it grow up to the surface of the water? If so, does it rise and fall with the water, or are the lower portions strong enough to support the upper portions of the plant? Or, does *Chara*, like various twining plants—*e. g.*, the morning-glory—depend upon other plants or upon some artificial support to hold it up? What is there about the aquatic habits of this plant which will explain its mode of growth? Can you detect any peculiar odor about *Chara*? If so, is it the odor of the water or of the plant?
- b. *Shape.*—Carefully separate from the mass a single plant and float it in a dish of water. What is its general shape? Can you plainly distinguish a

lower, or root end, from an upper, or stem end? How? Do you ever find roots except at one end of the plant; *i. e.*, does the plant ever take root at the joints?

*c. Size.*—Select four or five plants and measure their length. Do you get any very great variations in length? If so, how do you explain them? Any variations in diameter? How much of the length do you consider to be “root end”? How do length and diameter compare?

*d. Color.*—What is the general color of a single plant? Does the color vary in different portions of the plant body? If so, where is the color deepest? Where lightest? How do you explain these facts?

*e. Structure.*—Does the main stem bear appendages or **branches**? If so, do they originate at any particular points? How do they compare in general appearance with the main stem? Do the branches in their turn branch also? Note the circles or **whorls** of **leaves** on the stem. How do you distinguish leaves from branches? Where are the whorls most numerous? Do they occur at definite points? Do you find them on the branches? Are the leaves regularly arranged in the whorls? Do you find green and orange colored bodies, **reproductive organs**, on the leaves? Do you find these bodies elsewhere than on the leaves? Are the leaves subdivided into **leaflets**? If so, how do you distinguish the latter?

Note that the main stem consists of a series of segments or **internodes** which meet at joints or **nodes**. Do you find that the appendages like

wise consist of nodes and internodes? What relation exists between the appendages and the nodes? What variations in the length of the internodes do you find? Put a piece of the lower portion of a plant into dilute hydrochloric acid. What result? Try the tip end. Do you get the same result? Compare with the similar experiments on the exoskeleton of the lobster and on the mussel shell.

Make a drawing, showing the last six internodes and appendages.

*Microscopic Structure.*

- a. The **stem**.—With the hand-lens examine one of the upper internodes in water. Notice the elongated cells running in a spiral from the base to the apex of the node. How many turns does the spiral make? Does the internode have any outgrowths? Lay the same specimen on a slide and examine under the low power, using no cover-glass. Can you now trace the course of the spiral cells? Is each one continuous from one node to the next? If not, do you find that each spiral is made up of the same or of different kinds of cells? If the latter, how many kinds do you find, how do they differ from one another, and how are the various kinds arranged? Examine the lower portion of the stem and compare with the upper.

Draw two or three segments of the stem.

Hold a piece of a fresh or preserved stem between two pieces of pith, and with a sharp razor cut thin transverse and longitudinal sections through nodes and internodes. With a pipette

wash the sections into a watch-glass, place some of those made through the internode upon a slide with a drop of water, put on the cover-glass, and examine with the low power. Note that the section consists of a central or **internodal cell** surrounded by the **cortical cells**. How does the internodal compare with the cortical cells in size? In shape? In contents? Is the central cell divided? Does it communicate with the cortical cells? Does it extend through the nodes, or is there a single internodal cell for each internode? How many cortical cells are there? Do they have the usual contents of the cells of green plants, *i. e.*, protoplasm or primordial utricle, vacuole, starch grains, chlorophyll bodies, etc.? Note the **nodal cells** and compare them with the others. Compare several specimens to see if you can find any variations regarding the topics just studied.

Make drawings illustrating your results.

- b. **The leaves.**—With the scalpel cut loose from the stem two or three vigorous leaves, mount them in a drop of water, and examine under the low power. What is the shape of the leaf? Does it, like the main stem, consist of segments? If so, how many segments do you find in each leaf? Examine several leaves to see if the number is constant. Do you find nodal, internodal, and cortical cells? Are the latter arranged in spirals? Do these cortical cells differ in any important particular, as shape, arrangement, contents, etc., from those on the stem? Are cortical cells present on all of the internodes of the leaf? If not, which internodes lack them? Are these uncorticated inter-



nodal cells the same in position and number on all the leaves? What is the shape of the end cell of the leaf? Notice the leaflets. On what part of the leaf do you find them? Do they differ in any important details from the leaves? Are the leaflets arranged in whorls? Are all the leaflets at one node equally well developed? Do you find any relation existing between the leaves and the point of origin of the branches?

Put on the high power and examine the cells of the same leaf. Do you find that they have the usual cell contents? Note the chlorophyll grains. What is their shape? Size? Position in the cell? Do you find any part of the cell-wall which is not covered by the chlorophyll bodies? Are the chlorophyll bodies in contact with one another? Do you find any of them dividing? Are they stationary or do they move? What plant have you examined whose chlorophyll bodies most closely resemble these? Notice the thickness of the cell-wall. Notice the protoplasm moving in the end cells of the leaf. (This topic will be studied later under "Physiology.")

Make drawings of the structures of the leaf.

*c. The reproductive organs.*

1. The **antheridia**.—Select two or three leaves upon which may be seen, with or without a hand-lens, small, orange-colored bodies, the mature antheridia. On which leaves are the antheridia borne in the largest numbers? Examine with a low power. On what part of the leaf do you find them? What is their relation to the nodes? To the leaflets? Are the antheridia situated with

reference to any particular leaflets? If so, which? Why are they so situated? What is the shape of an antheridium? To what portion is the color confined? How is the surface marked? By comparing a number of antheridia which lie in different positions try to make out that the surface of each is divided into eight areas, the **shields**. The centre of each shield is indicated by a circular cell. From this radiate oblong cells, which unite in a dentate manner with similar cells forming parts of neighboring shields. The four shields covering the outer end of the antheridium are somewhat square in outline; the other four, at the base of the antheridium, are nearly triangular. With a dissecting-needle tap gently on the surface of the cover-glass, and try to separate an antheridium from its point of attachment. Can you detect a **stalk**? Carefully press upon the cover-glass until the antheridium breaks. Examine with the high power one of the shields. To what is the color of the antheridium due? Notice projecting into the centre of the sphere from the central cell, seen on the outside, a single club-shaped cell, the **manubrium**. If the antheridium is not too deeply colored, these manubria may frequently be seen in their natural position without crushing the antheridium. On the end of the manubrium find a rounded cell, the **capitulum**, to which are attached several smaller cells, the **secondary capitula**. From these grow a number of elongated filaments. What is the shape of these filaments? Color? What is their structure? Estimate the number of cells in one filament. Try to find in each cell a spiral

**spermatozoid.** If the antheridium be quite mature, some of the spermatozoids may be seen swimming about in the water. If so, examine them carefully. What is their shape? Do they have the same diameter at each end? At their anterior end look for two long **flagella**. If the structure of the spermatozoid is not plain, apply dilute iodine. How do the spermatozoids move? For how long is their motion kept up? Look for antheridia in different stages of development. Study and draw the stages found.

Make drawings showing: (1) the antheridium as seen from the outside; (2) a shield seen from without; (3) a shield with manubrium, etc.; (4) a spermatozoid.

2. The **oögonia**.—Compare these as regards position, shape, size, color, etc., with the antheridia. Do you find any constant relation existing between the position of the antheridia and that of the oögonia? Is the oögonium borne upon a stalk? Note its outer covering of spirally twisted cells. Do these run as single cells from the base to the apex of the oögonium? How many are there? Note the **crown**, consisting of small cells. What is their number? What is their position with regard to the spiral cells? Note the central cell or **oösphere** of the oögonium. How does it compare in size with the other cells? What does it contain? Certain points in the structure of the oögonium may be found to be more easily seen in specimens preserved in chromic acid and examined in glycerine.

Draw an oögonium, showing its entire structure.

Examine a number of specimens for oögonia in

various stages of growth. Do you find any, especially the very young oögonia, in which the outer cells have not yet taken the spiral form? Do you also find some in which the spiral cells have become dark-colored and hard, the **ripened oöspores**?

Draw all of the stages found.

- d. The **terminal bud**.—With scissors cut off the upper portions of several plants, put them into one per cent. chromic acid for a day, then remove one of the terminal buds to a drop of glycerine on a slide; with dissecting-needles pick away all of the lower parts of the bud, remove these, and put on the cover-glass. While examining the bud under the low power gently tap and press upon the cover so as to push away all of the undeveloped leaves which cover the apex of the stem. How are the leaves arranged in the terminal bud? Can you trace nodes and internodes in the bud? At the very tip of the main axis find the **apical cell**. What is its shape? Does it have the usual cell contents? How many nuclei does the cell contain? Do you find a single cell, the **segmental cell**, just below the apical cell? By tracing back from the apex endeavor to see how the nodal and internodal cells are formed, and how the nodal cells eventually give rise to the cortex and to the leaves.

Make drawings to illustrate your observations.

- e. The **rhizoids**.—Examine the nodes and the lower end of the main stem of living plants for the flocculent clusters of root-like appendages, the **rhizoids**. Do you find them on the internodes? On

the leaves? At *all* of the nodes? Are all the rhizoids surrounded by masses of mud? Examine under the low power. What is the arrangement of the rhizoids borne at one point on the stem? Are they arranged in whorls? Do they originate from particular cells? If so, from which cells? Do two or more rhizoids originate from the same cell? Put on the high power and study a single rhizoid. What is its shape? Does it vary in diameter? Is it segmented? Does it have a layer of cortical cells? Of how many cells does it consist? What are its contents? Note the circulation of the protoplasm, which will be studied later.

Draw several rhizoids, showing their shape, structure, mode of attachment to the stem, contents, etc.

If specimens of *Nitella* can be obtained they should be compared with *Chara*.

#### PHYSIOLOGY

*a. Growth.*—Put a single vigorous plant into a dish of water. With a pair of dividers accurately measure each internode, beginning at the lower end of the plant, and record the measurements. What is the length of each internode and of the entire plant? Place the dish in a window where the plant may have plenty of sunlight. At the end of a week measure all of the internodes again and record the measurements. Do this for two or three weeks in succession. How much has the plant grown in length in this time? Is the increase due to the formation of new internodes or to the lengthening of those already formed at the beginning of the experiment or to both? If to

the first, where have the new internodes formed? Do you find that new internodes form *between* the others? If growth is due to the lengthening of the internodes, which have lengthened the most? Do you find that your results hold good for the branches as well as for the main stem? Does the diameter of the stem increase also? Note the liberation of bubbles of oxygen from plants exposed to the sunlight. Compare with *Spirogyra*.

- b. *Movements of protoplasm*.—With a high power examine the terminal cells of a leaf of *Chara*, or, better, an entire internode of *Nitella*, and note the moving protoplasm. In what part of the cell is it seen? What is the direction of the flow? Examine several cells to see if this is uniform. Is the rate of flow the same in all cells? Is it uniform in all parts of the same cell? Are the chlorophyll bodies carried along by the stream? Does the stream flow from one cell into another? Can moving protoplasm be found in all of the cells of the same plant? If not, in which cells is it found? What is the color of the protoplasm? Note the granules carried along by the current. How long does it take a granule to make the circuit of the cell?

Examine in like manner some of the rhizoids, and compare the moving protoplasm in them with that in the leaves.

*General Questions*.—Is the plant so firmly attached by its roots to the mud in which it grows, and is the root-system so well developed, as to warrant thinking that *Chara* absorbs a large part of its nourishment through

its roots? If not, how does the plant probably get its nourishment? Is it like *Spirogyra* and *Vaucheria* in this respect? Of what use is the limy coating? Do you find that, aside from the reproductive organs, there is any great variety of shape, size, structure, etc., exhibited by the cells of *Chara*? What structural resemblances do you find to exist between leaf and stem? What differences in their terminations?

Break the branches off a vigorous plant, put them in water, and watch them for several weeks. Do they continue to live? If so, could *Chara* be propagated in this way, *i. e.*, without the formation of spores?

Do you find any points of resemblance between *Chara* and *Vaucheria*?

Does the general plan upon which *Chara* is constructed, *i. e.*, a segmented body with segmented appendages, remind you of any *animal* which you have studied?

What do you regard as the most striking feature of the cells of *Chara*?

Compare *Polysiphonia*, one of the red sea-weeds, with *Chara*.

### **Rockweed (*Fucus Sp.*)**

*Material.*—Rockweed is to be found almost everywhere along the coast, attached to the surface of rocks, timbers, shells, etc., or carried along by the currents or cast ashore. It can be sent on a several days' journey inland and arrive in good condition, if care be taken to pack it in a close wooden or tin box. On arrival it should be placed in real or artificial sea water, if a prolonged study is to be made. Otherwise, it may be kept in good condition if laid in a cold, damp place. It should not, however, be moistened directly with fresh water. In many towns it is possible to get good material from restaurant keepers and fish-dealers, who receive the rockweed as packing around lobsters, crabs, etc. For the study of structure, alcoholic is better than fresh material. The plants may be put directly into alcohol, beginning with fifty per cent. and passing through the various grades, leaving the specimens from twelve hours to a day in each grade; or, they may be placed for two to four hours in a mixture consisting of one part of a saturated solution of picric acid in sea water to four parts of sea water; then, after washing for about fifteen minutes in sea water to remove the excess of the acid, passed through the different grades of alcohol.

Additional requisites consist of the compound microscope, hand-lens, fine forceps, scalpel, razor, watch-glass, pipette, glycerine, sulphuric acid, Schulze's solution, glacial acetic acid, and pith.



*Method of Examination.*—Laboratory specimens, if living, should be studied in sea water; if alcoholic, in fifty per cent. alcohol. Sections are most easily cut from the preserved specimens and must be examined in a mixture of glycerine and fifty per cent. alcohol. Sections of fresh material should be studied in sea water or in a mixture of three parts of salt to one hundred parts of fresh water. Inland students may sometimes be fortunate enough to see the extrusion of the sexual cells, if plants gathered at high tide be sent them, accompanied by a supply of sea water. On receipt of the plants they should be divided into two sets. Place the first set in the sea water. Hang the second set in a cool place. Leave both sets for about six hours. During that time some of the hanging plants will probably have extruded their sexual cells, the male cells or **antheridia** being orange-yellow, the female cells or **oögonia** olive. Then hang up the plants which have been in water, and place the hanging plants in the water for about six hours. This may be repeated a great many times.

#### MORPHOLOGY

##### *Naked-eye Characters.*

- a. *General appearance.*—If possible study the plant as it grows, attached to the face of a rock or to the surface of a timber. Note its relation to the tide marks, its rise and fall with the tide, etc. Dig down into a mass of the plants left exposed by the fall of the tide, and note the differences between those plants which are on the surface and those deep down in the mass. Then examine an entire fresh or preserved specimen. What is the general shape of the plant-body, or, as it is

called in this case, **thallus**? What is the color of the thallus? Does the color vary in different places? Does the plant apparently contain chlorophyll? Place a plant in sixty per cent. alcohol for a few hours. What change in the color of the alcohol? In the plant? Is the plant of sufficiently firm structure to stand upright by itself? Can you distinguish an upper and a lower side on the thallus? The plant consists of an attached portion or **disk**, from which proceeds the **stem**, the latter dividing into flattened **branches**.

- b. The **disk**.—Shape of the disk? In structure which does it resemble the more closely, the stem or the branches? By what means is the disk attached? Is it very firmly attached? Is the plant attached by any other part than the disk? Do you ever find more than one stem growing from the same disk? Is the disk merely an organ of attachment, or does its appearance warrant regarding it an organ of absorption, or root, as well?
- c. The **stem**.—Where does the stem begin? Is there any sharp distinction between disk and stem? What is the shape of the stem? Does it on its lower part bear any wing-like expansions?
- d. The **branches**.—Trace the branches of the stem up into the expanded portions or **branches** of the thallus. Here they form the **midribs** of the flat branches. How do you distinguish the midrib from the other parts, **wings**, of the branch? What part of the branch does the midrib occupy? Does each branch have a midrib? If so, does it extend to the end of the branch? Note the

manner in which the midrib subdivides. Is it regular? Are all of the branches flattened in the same plant? Note at certain points on the thallus of some of your specimens smooth swellings, the **vesicles**. Whereabouts on the thallus are these most numerous? Are they arranged in a definite manner with regard to one another? With regard to the branching of the midrib? Are they thickenings of the midrib or of the wing? Pinch one of these swellings between the thumb and the finger. Is the swelling hard or soft? Can you easily break it by pinching? With the razor cut one of the vesicles in two. What do you find inside it? What is the nature of its wall? Being careful not to injure the vesicle, cut off a portion of the branch about an inch long, containing one or more vesicles, and throw the piece into salt water. Does the piece float or sink? Try the same experiment with another portion of the branch free from vesicles. What result? What is the use of the vesicles in a plant which grows attached to a solid substratum?

Examine the tips of some of the branches. Do you find them swollen and covered with tufted points, the **conceptacles**? Do these conceptacles have a definite arrangement? Do you find them elsewhere than on the tips of the branches? Do they occur on the midribs? On both sides of the branch? Pinch such a tip. Is it as firm and hard as the rest of the thallus? Examine some of the conceptacles with a hand-lens. What is the cause of the tufted appearance? Can you find in the centre of each tuft an opening, the **ostiole**, which leads into the cavity of the concep-

tacle? With a scalpel cut open the swollen end of a branch. What is the appearance of the inside?

Sketch a plant and show all of the features observed. Examine the tips of branches which are not swollen. Do they likewise bear conceptacles? Note that the extreme tip is indented. In the bottom of the depression lies the **growing point**. Put a piece of the thallus into fresh water. Does the piece gradually become slimy? Does it increase or decrease in size? Does it go through these changes in salt water? Why must specimens be studied in salt water, or in alcohol, or in glycerine and alcohol?

*Microscopic Examination.*

- a. The **branch**.—Hold a piece of a young branch of a preserved specimen between two pieces of pith, and with the razor cut both transverse and longitudinal sections, and examine in glycerine with the low power. What is the shape of the outline of the branch? Notice the arrangement of cells in the section, certain cells forming a surface or **cortical band**, which shades off through a layer of **parenchyma** into a central mass or **medulla**. Do the former resemble the cortical cells of *Chara* in arrangement, shape, size, color, etc.? Is the position of the midrib well defined? Put on the high power and examine the same section. Study the cortical cells. How are they arranged? How far towards the middle of the branch do they extend? Are they sharply marked off from the underlying tissue? What is their color? Do you find the same color elsewhere in the section?

What is their shape? Are there any marked variations in shape? Do they differ much in size? Does their wall vary much in thickness? If so, where is it thickest? What do the cortical cells contain? Do you find starch? Are their contents more or less abundant than those of cells lying near the middle of the thallus? If so, can you explain why? Examine the layer of parenchyma which lies between the cortical cells and the medulla. Is this layer sharply defined? What is the general shape of these cells? Are they well filled with contents? Is starch present? Note that the walls of many of the parenchymatous cells are thickened and marked with circular, oval, or irregular dots, the **pits**. Study the medulla. What is its color? Notice how exceedingly thick the cell-walls appear. How do the cavities of the medullary cells compare in shape and size with those of the cortical and parenchymatous cells? What do the medullary cells contain? Test for starch. Is it present? Place a section in a drop of fresh water and watch the changes in the medulla. What happens? Do your observations on this section explain the changes seen when a portion of the thallus was placed in fresh water? Apply a drop of strong sulphuric acid, which dissolves cellulose, to a section. What happens to the cells of the medulla? To the cortical cells? Do the walls of the cortical cells, particularly the outer walls, consist of cellulose? To another section apply a drop of Schulze's solution. What change in the walls of the medullary cells? Do you find an **inter-cellular substance** which is not cellulose?

Does this experiment confirm the result obtained with sulphuric acid? What effect has Schulze's solution on the outer wall of the cuticular cells?

Draw the section as seen under the low power, showing the shape of the branch and the position of the various tissues. Make another drawing of a portion of the section seen under the high power, and showing the structure, etc., of the various cells. Make longitudinal sections of the young branch and compare them with the transverse sections. Draw.

Examine as above transverse and longitudinal sections of the older branches and of the stem.

6. **The growing point.**—Some of the longitudinal sections of the young branch made above probably pass through the notch at the end of the branch. If not, make such sections, mount in a drop of a mixture of equal parts of glycerine and glacial acetic acid, and examine under the high power. What is the shape of the notch? Of the tips of the branch on each side of the notch? Note that the notch contains a clear mucilaginous substance. Does this entirely fill the depression? Does it extend around on the tips of the branches? If so, how far? Of what use may it be? Note the group of cells, the **initial cells**, at the bottom of the notch. How are they arranged? What is their relation to the surrounding cells? Notice the large central cell, the **initial** or **apical cell**. What is its position with regard to the other initial cells? To the bottom of the notch? What is its shape? Size as compared with the neighboring cells? Can you make out the manner in which this cell di-

vides to form the surrounding tissues? Trace back into the older portions of the branch the rows of cells which have their origin from the initial cells. How do these tissue-cells change in shape and contents as they get older?

Draw your section.

Make transverse sections across the young branch, cutting through the growing point as well as above and below it.

Compare with the longitudinal section and draw.

c. **The vesicles.**—Examine transverse and longitudinal sections of the walls of the vesicles. Do you find all of the tissues present? Look for vesicles in various stages of development. Judging from their structure, how do you imagine the vesicles to be formed? Draw the sections.

d. **The conceptacles.**

1. **The sterile conceptacles.**—With a hand-lens search among your sections for some which have passed through the conceptacles. Mount several of these sections in a drop of the mixture of glycerine and acetic acid, examine with the low power, and find a section which has passed through the centre of a conceptacle. What is the shape of the cavity? How is it formed? Is the cavity apparently anything more than a depression of the surface of the thallus? How much of the width of the section (thickness of the thallus) does a conceptacle occupy? Notice the cluster of **hairs** or **trichomes** in the conceptacle. What is their relation to the opening or **ostiole** of the conceptacle? What part of the conceptacle forms the

elevation noticed on the surface of the thallus? Examine under the high power. From what part of the conceptacle do the hairs grow? What is their structure? Are they simple or branched? What tissue forms the wall of the conceptacle? Do you find anything but hairs in these conceptacles? Examine both transverse and longitudinal sections to get views of conceptacles from different directions. Look over your sections for conceptacles in various stages of formation. Are they formed in the same manner as vesicles? Draw.

2. The **male conceptacles**.—With a razor cut transverse sections through the end of an alcoholic branch bearing male conceptacles, and examine under a low power. What is the shape of the section? What sort of tissue occupies the centre? The edge? In what part of the section do you find the conceptacles? Does their position correspond with that of the sterile conceptacles? Do you find the same parts, ostiole, hairs, etc., as in the sterile conceptacles? How do the male compare with the sterile conceptacles in size? Draw.

Study the sections under the high power. Examine again the tissue composing the centre of the section. Of what kind of cells is it formed? How are they joined together? What do they contain? Do these cells entirely fill the centre of the section? Can you now account for the feel of the end of the branch when passed between the fingers? Examine the hairs. Are those which fill the cavity of the conceptacle at all different from those which project through



the ostiole? Notice attached to the former small ovoid cells, the **antheridia**. To what part of the hairs are the antheridia attached? Do you find more than one antheridium borne on a single hair? Notice the contents, the **antherozoids**, of the antheridia.

Draw a conceptacle as seen under the high power. Draw also several hairs with their attached antheridia, and some of the cells forming the central tissue.

3. The **female conceptacles**. — Prepare sections showing female conceptacles. Study these as directed above, and compare the sections in every respect with those containing the male conceptacles. Compare the female with the sterile and the male conceptacles as regards form, size, contents, etc. Examine especially the **oögonia**. What is their shape? How do they compare in size with the antheridia? Are they borne upon hairs like the latter? If not, how are they held in place within the conceptacle? How does the number of oögonia compare with the number of antheridia in a conceptacle? Has each oögonium a covering? If so, what is its structure? Can you detect any outgrowths, *e.g.*, **cilia**, on it? Do you find the contents of any of the oögonia to be divided in several parts or **oöspheres**? If so, how many such parts can you distinguish in a single oögonium? Does each part have a nucleus? Is each part a cell? Mount a section in a drop of fresh water. What happens to the coverings of the oögonia?

How are the male and the female conceptacles distributed in the same or in different plants? What is their relation to the sterile

conceptacles? Do you ever find oögonia and antheridia in the same conceptacle?

Draw the section as seen under the low power, and the conceptacle and a single oögonium as seen under the high power.

#### PHYSIOLOGY

- a. *The fertilization of the oösphere.*—From plants that have been hanging in the air collect a drop of the orange-colored fluid that exudes from the male conceptacles. Mix this drop with a drop of sea water and examine under the high power. Note the **antheridia** floating in the drop. Look for **antherozoids** actively swimming about. What is their shape? Color? Structure? By what means do they swim? Apply a drop of iodine if necessary. Are they at all like the antherozoids found in *Chara*? Draw.

Mount in a similar manner a drop of the olive-colored fluid issuing from the female conceptacles. Look for **oögonia**. Try to find an oögonium whose wall is dissolving, thus setting free the **oöspheres**. Do the oöspheres change in shape before being liberated? What is their structure? Can you detect a nucleus? Have they any means of locomotion? Draw.

After finding some mature oöspheres, mix on the slide with the water containing them a drop containing antherozoids. Study the behavior of the antherozoids and try to see as much as possible of the union of two kinds of cell (*fertilization*). Draw.

*General Questions.*—Are the methods of reproduction of rockweed likely to lead to its wide distribution? Compare with *Chara* and *Vaucheria* in this respect. Is the plant structurally adapted to endure long exposure to the air without serious injury?

### **Mould (*Penicillium* Sp.)**

*Material.*—Examine stale bread or cake, shoe-blackening, old leather, ink, preserves, etc., for the common blue mould. If none be found, specimens may easily be raised by moistening a piece of bread, covering it with a bell-jar, and allowing it to stand in a warm place for about a week. At the end of that time the bread will probably be covered by a fine crop of moulds, and among them the kind wanted. A decoction of prunes may be left standing exposed to the air for a time, and *Penicillium* will almost certainly make its appearance.

Schulze's solution, iodine, potash, Pasteur's solution with sugar, watch-glass, fine forceps, hand-lens, compound microscope, moist chamber, twenty per cent. glycerine, and thirty per cent. alcohol will be needed.

*Method of Examination.*—Look for *Penicillium* growing naturally. Try to find how many different substances it infests. Compare specimens developed naturally with those raised by cultivation.

### **MORPHOLOGY**

#### *Naked-eye Characters.*

- a. *General appearance.*—Examine the coating of mould found on the infested material. Do you find the mould to be in several masses or in one? What is the color of the mould? Do you find any variations of color? If so, do the various colors shade

into one another? Do these different shades have any definite relation to the centres of the masses of mould? How high above the surface does the mould rise? Can you with a lens distinguish the individual stalks or **aërial hyphæ**, each with a tuft-like head, the **conidiophore**? Examine with a lens and note the root-like threads forming the **mycelium**, which runs over the surface of the substratum. With the fine forceps pick up a small mass of mould. Is it attached to the substratum or is it merely lying on the surface?

Tap a piece of mouldy bread and notice the cloud of dust, the **spores**, given off.

*Microscopic Characters.*

- a. The **mycelium**.—With the fine forceps place some of the mycelium on the slide in a drop of water, twenty per cent. glycerine or thirty per cent. alcohol. Does water readily wet the mould? Using fine dissecting-needles, gently pick to pieces the mass of mould, and examine under both the low and the high powers. What is the structure of the mycelium? Do the threads, the **mycelial hyphæ**, branch? If so, are the branches given off at definite points? Do the hyphæ consist of a single cell or of several cells? Do the hyphæ vary in diameter? What is their shape at the end? What is the structure of the cell-wall? What do the hyphæ contain? Examine mycelium of the different colors. What variations in structure and state of development do you find?

Make several drawings to illustrate the various arrangement of the mycelial hyphæ and their structure.

- b. The **aërial hyphæ**.—At what points do the aërial

hyphæ originate? How do they compare with the others in shape, size, structure, etc.? Do they branch? If so, at what points? Do you find more than one branch given off at one point? Do the cavities of the aërial connect with the cavities of the mycelial hyphæ? Are the cavities of the branches in communication with the cavity of the main stalk?

- c. The **conidiophores**.—Study the structure of the terminal branches or **conidiophores**. Note that each tapers to a short, slender stalk, or **sterigma**, which bears a series of spherical or oval spores, the **conidia**. Draw. Study a single stalk of conidia. Does it branch? Do you find any variations in shape, size, and color among the conidia of the same stalk? If so, what position on the stalk do the larger conidia occupy? Do you find any conidia which are not yet fully formed? If so, what is their position on the stalk? Judging from the facts just observed, what do you think is the manner in which the conidia are formed? Study the structure of a single spore, using such reagents as are necessary. Does it resemble in any way the cells of yeast? Draw.
- d. The **sporocarp**.—It is not likely that the student will be able to find this organ on *Penicillium* and work out its structure successfully. The sporocarps of *Eurotium*, which usually grows with *Penicillium*, may be used instead. These will be found as small, bright-yellow bodies situated among the mycelial threads. Examine one of them under both powers. Note how it is attached to the mycelium. Study the structure

of the outer wall. Draw. Crush the wall by pressing upon the cover-glass and notice the contained sacs, the **asci**. How many does a single sporocarp contain? What is the shape of an ascus? What is its structure? How many spores, the **ascospores**, does each ascus contain? What differences can you detect between these spores and the conidia? Draw an ascus with its spores. Look for sporocarps in various stages of growth. They develop from two branches which become twisted around each other in the form of a short spiral. Draw all of the stages found.

#### PHYSIOLOGY

##### *a. The germination of the conidia.*

Prepare a moist chamber, using a drop of Pasteur's fluid with sugar for the hanging drop. With the fine forceps pick up a few aerial hyphæ with conidiophores, and sweep the conidiophores over the surface of a drop of water on a slide so as to brush off some of the conidia into the drop. Then dip the point of a needle first into the drop of water and then into the hanging drop, thus transferring a *few* conidia to the latter. Examine the conidia from time to time for three or four days. How long before you can detect signs of germination? What is the first change noticed? How many hyphæ does each spore form? Do these hyphæ branch? Do they interlace to form a mycelium? Do the mycelial hyphæ unite?

Draw the various stages of germination.

*General Questions.*—How do you account for the very wide distribution of blue mould? For its occurrence in cans of preserves?

### **Mushroom (*Agaricus Sp.*)**

*Material.*—Mushrooms are usually abundant in pasture lots in the late summer and early fall. They frequently grow in gardens. In greenhouses they may be found at various times during the year. They may often be obtained from gardeners who raise them for the city trade. In case mushrooms cannot be obtained, almost any of the common toadstools will do as well. Get specimens showing as many different stages of growth as possible. Both fresh and alcoholic material will be used. The latter is prepared by hardening the mushrooms in one per cent. chromic acid for a day, washing off the superfluous acid in fresh water for two or three minutes, then placing them for about twelve hours in each of the following grades of alcohol—forty, sixty, seventy-five, and ninety per cent. A part of the alcoholic material may be examined entire and the rest of it sectioned. Specimens to be sectioned should be cut into pieces not more than a half-inch square before being placed in the chromic acid.

Apparatus and reagents required: dissecting-needle, bell-jar, watch-glass, compound microscope, lens, razor, fifty per cent. glycerine, acetic acid, hydrochloric acid, carmine, and Schulze's solution.

*Method of Examination.*—If the student have access to living specimens growing naturally, let him study their surroundings, the kind of soil upon which they



grow, their mode of attachment to the soil, the number of individuals growing together, etc., etc. Specimens may then be removed to the laboratory and studied without and with the microscope. Preserved material should be examined in a dish of seventy-five per cent. alcohol.

#### MORPHOLOGY

*Naked-eye Characters.*—Select several mushrooms or toadstools, and study

- a. *Shape.*—What is the general shape? Do you find any marked variations from this? Do you find that the mushroom always has the same shape as the mature specimen? If not, what changes of shape does it go through during its development?
- b. *Size.*—What is the height of a mature specimen? What variations do you find? Does the mushroom attain its mature form before or after it reaches its full height?
- c. *Structure.*—Examine a mature specimen and make out the following parts, the expanded top or **pileus**, and the stalk or **stipe**, surrounded by a ring, the **annulus**. Study each part in succession.
  1. The **pileus**.—What is its shape? Diameter? Thickness? Color of the upper surface? What is the structure of the upper surface? Can you give any reasons for this structure? Examine the extreme margin of the pileus for a thin membranous expansion. What is the color of the under surface? Note that it varies from pink in young to dark-brown or black in old specimens. Note also the **gills** or **lamellæ**. How are they arranged? What is their shape?

Structure? Color? Cut vertically through the middle of the pileus. What is the nature of the inside?

Draw the pileus as seen from above, from below, and in section.

2. The **stipe**.—What is its shape? Does it have the same diameter throughout? What is its color? Does it have the same color in all specimens? How is it attached to the pileus? Is it attached to the gills? Examine the lower end of the stipe. How is it attached to the soil? Can you find any roots? Use a hand-lens and look for the remains of the **mycelium**, from which the aërial part of the plant (the “mushroom” proper) arises. As the mushroom develops, does the stipe increase the more in diameter or in length? Make transverse and longitudinal sections through the stipe, and compare its structure with that of the pileus. Draw.

3. The **annulus**.—What is its shape? How far from the pileus does it grow? Examine its structure, and compare it with that of the membrane on the margin of the pileus. Examine a series of specimens to see that the annulus and the membrane are at one time connected, and form the **veil** or **velum**. At what time and how do they become separated? Has the velum any definite function? Study a series of specimens, and draw the different stages of development.

#### *Microscopic Structure.*

- a. The **mycelium**.—Remove from the soil a young

mushroom, leaving some of the earth adhering to the mycelium. Cut off the lower end of the stipe and place it with the mycelium in a watch-glass of water. Carefully wash and pick away as many as possible of the earthy particles, place the preparation on the slide in a drop of water or of dilute glycerine, and examine. Notice the white threads visible without the microscope. Are these bands of hyphæ or individual threads? Note the interlacing of the mycelial hyphæ. What is the structure of these? What do they contain? Look for rod-like crystals. Are they within or without the hyphæ. Test these with acetic acid. Do the crystals dissolve? If so, they consist of carbonate of lime. If the crystals do not dissolve in acetic, test them with hydrochloric acid. If they dissolve, they consist of oxalate of lime.

Endeavor to make out how the lower end of the stipe is attached to the mycelium. Can you trace mycelial hyphæ running into the stipe?

Draw several portions of the mycelium to show its structure and arrangement.

- b. The **stipe**.—Make a longitudinal section passing through the middle of the stipe and the annulus. Examine under a low power. Can you distinguish tissues in the stipe? Is its centre more or less dense than its margin? From what part does the annulus arise? Put on the high power. Notice that the stipe consists of rows of hyphæ, each hypha being divided into a number of cells by cross walls or partitions, the **septa**. In what direction do these rows run? Do they interlace to

any great extent? Do they branch? Examine the individual cells. What is their shape? Arrangement? Structure of their walls? What do the cells contain? How do those near the centre of the section compare in diameter with those at the margin? Does the annulus have the same structure as the stipe? In what direction do the end walls of the cells run? Compare a single row of cells with a hypha. Is the stipe anything more than a mass of hyphæ? Stain the section with Schulze's solution. Note the color assumed by **fungus cellulose**. Is it the same as ordinary cellulose? Do the cells contain starch? Draw your sections, showing all of the features observed. Make a transverse section of the stipe, and examine in dilute glycerine. Compare with the longitudinal section. Note the large, nearly circular, **intercellular spaces**. Where are they most numerous? What is the shape of the hyphæ in trans-section? Do they vary in diameter? If so, where are the largest? Notice that some of the hyphæ show in the centre a highly refractive spot. Can you find any trace of this on the end walls in the longitudinal section of the stipe? Draw.

- c. The **pileus**. — Make a vertical section passing through the middle of the pileus and of the stalk, and examine under a high power. Is the structure of the mass of the pileus the same as that of the stipe? What course do the hyphæ take upon entering the pileus? Do the hyphæ extend into the gills? Make a diagram showing the course of the hyphæ through the entire plant. Make

another vertical section passing through the pileus to one side of the stipe and through the gills in such a way as to divide the latter transversely. Examine under the low power. How are the lamellæ attached to the pileus? Do their "tissues" seem to be continuous? Draw. Examine under the high power. Endeavor to make out the central portion, or **trama**, the **sub-hymenial layer**, and the outer portion, or **hymenial layer**, of each gill. What is the structure of the trama? In what ways does it differ from the central part of the stipe and the pileus? What is the course of the hyphæ? How do you distinguish the sub-hymenial layer? How do its cells differ from those of the trama? What is the structure of the hymenial layer? What is the direction of its cells? Are they continuous with those of the next layer below? Study this layer closely, and endeavor to make out that it consists of two kinds of cells, those with rounded ends, the **paraphyses**, and the **basidia**, ending in two pointed processes or **sterigmata**. How are the paraphyses and basidia arranged with regard to each other? Can you think of any use which the former may have? How do they differ in shape? Note the rounded body, the **basidiospore**, borne by each sterigma. Examine sections from young mushrooms to find the paraphyses, basidia, and spores in various stages of development. Note how the sterigma and its spore are formed. Make a diagram showing the arrangement of the tissues of the lamella. Draw several isolated paraphyses and basidia.

Break off the pileus of a mushroom whose

gills are dark-brown or black, lay it, gills downward, on a piece of white paper, and cover with a bell-jar or tumbler. After five or six hours remove the pileus, and note the "print" of its under surface made upon the paper by the spores. To what do the dark lines correspond? To what the white? With a needle place some of these spores in a drop of water, and examine under the high power. What is their shape? Structure? Color? Draw several. Pull off a single gill and lay it on a slide without the drop of water and cover-glass. Note the spores, many of them detached from the sterigmata. What is the color of the tissue of the gill? To what is the color of the under side of the mushroom due?

*General Questions.*—Review the whole structure of the mushroom. Do you find the aërial fruiting portion to be anything more than a collection of hyphæ, at the tips of some of which spores are borne? In what various ways do the blue mould and the mushroom resemble each other? What do you consider to be essentially their points of difference? Can the blue mould and the mushroom obtain their food in the same manner as green plants? Are they dependent upon the sunlight?

### **Liverwort** (*Marchantia Sp.*)

*Material.*—Though this plant may frequently be found growing in the grass or on rocks in damp, shady places, especially near springs, or where the water is dripping down a stony embankment, it is most easily obtained at the greenhouses. It grows on the soil in the rose-beds, and the various stages may be found at one time. Good specimens bearing the three kinds of fruiting organs are generally abundant in the fall and in early spring. The male plants with their flat-topped umbrella-shaped stalks usually grow near the female plants, which may be distinguished by the star-shaped tops which the fruiting stalks bear. When a good supply of fruiting specimens is found they should be preserved in alcohol, for fear that fresh ones may not be obtainable when wanted. Both fresh and alcoholic material will be studied. Living specimens should be removed to the laboratory along with plenty of the earth to which they are attached. Place them in flower-pots or dishes filled with loose, damp soil, and cover with a plate of glass or a bell-jar. Do not place the plants where the sun is too hot, as they are very likely to be “scorched” and killed.

Besides the specimens the student will need the compound microscope, hand-lens, razor, forceps, watch-glass, pipette, fifty per cent. glycerine, Schulze’s solution, iodine, pith, and dissecting-needles.

*Method of Examination.*—Study the plant as it grows

in the grass, on the rocks, or in the greenhouse. Notice what sort of soil it occupies and the general character of its surroundings. Examine the gross anatomy of the plant, then make sections and study its internal structure.

#### MORPHOLOGY

##### *Naked-eye Characters.*

*a. General appearance.*—Examine first a plant bearing none of the upright stalks which produce the sexual organs. What is the general shape of the body (**thallus**) of the plant? What part of the thallus is in contact with the ground? Does the thallus present well-marked dorsal and ventral sides? Compare with rockweed. What is the color of the thallus? Width? Thickness? Tear a thallus to pieces and note its texture. Does it have a midrib? Compare with rockweed. Does the thallus bear horizontal branches? If so, in what manner are they given off? Compare with rockweed. Do certain parts of the thallus appear to be older than others? If so, where are these parts? Compare with rockweed. In which direction does the thallus grow? Compare with rockweed. Examine next a plant bearing the upright fruiting stalks. At what points do these stalks grow? Is this position constant? Aside from the stalks, do these plants differ in any essential particular from those which are without stalks? Examine a number of plants to find the two kinds of fruiting stalks, the male or **antheridial branch**, with a flat, and the female or **archegonial branch**, with a star-shaped expansion at the top of each stalk. Do the male and



female stalks occur on the same plant? Are they always borne upon the same side of the thallus? Do you always find the same number on each plant? What is their relation to the midrib? What is the shape of the stalk of the fruiting branches? Look for **grooves** running lengthwise along the stalk. How many do you find? What is their position? Examine both surfaces of the expanded portion or **receptacle** of each. Note the ridges on the receptacle of the antheridial stalks. How many are there? Are they on both sides? Compare in all respects with the archegonial branches. Look for the two kinds of fruiting branches in different stages of growth. What changes in shape and size do they go through in the course of their development?

Look closely at the upper surface of the thallus for small, cup-like outgrowths, the **cupules**. What is their position? Does it have any particular relation to the midrib or to any other part of the thallus? Are the cupules evenly distributed over the surface? What is their shape? Look for small, green, non-sexual reproductive bodies, the **gemmae**, inside the cupules. Do all of the cupules contain gemmae? Are the cupules borne upon a stalk? Are they borne upon the same plants as have the fruiting branches? Do you also find cupules upon plants which bear no fruiting stalks? With a hand-lens examine a cupule and note the shape of its margin and the position of the gemmae. With a lens study the upper surface of the thallus and note that it is marked off into small areas, the **areolae**. In

the centre of each area look for a small circular opening, the **stoma**. Examine the under surface of the thallus for root-like filaments, the **rhizoids**. Are they abundant or few? Do they grow from any definite area of the under surface? Do they grow at the tips of the branches? What is their average length? Do they hold the plant closely to the soil? What is their color? Do they at all resemble the rhizoids seen on *Chara*? Look among the rhizoids for purple leaves following the line of the midrib. Do you find areolæ and stomata on this surface?

Make sketches of plants showing all of the features studied.

*Microscopic Structure.*

- a. The **tissues of the thallus**.—Hold a piece of the thallus of a living plant between two pieces of pith, and with a sharp razor cut a series of thin, transverse sections. Lay several of these on a slide in a drop of water or fifty per cent. glycerine and examine with the low power. If it is found that the tissues of the living plant contain so much air as to obscure the structure, use the sections of the fresh specimens only to get a general idea of the structure, then work out the details in alcoholic material. Notice the shape of the section. How do you locate the midrib? Notice also that the section shows its upper surface to be composed of a layer, the "**epidermis**," of clear cells showing elevations (sections of stomata) here and there. Below this layer comes another consisting of dark-green cells, and this is succeeded

by a rather thick mass of large, clear cells, some of which contain brown oil globules. Notice also the rhizoids.

Make a diagram showing the position of all these parts. Put on the high power and study these parts separately.

Of how many layers of cells does the "epidermis" consist? What is their shape as seen side-wise? Do they contain chlorophyll? What is their arrangement at a stoma? Notice that air-bubbles are frequently present in the tissues, especially under the stomata. What is the shape of the cells in the green band? Are they closely or loosely packed together? Is their green color due to the fact that their chlorophyll bodies are larger or that they are more numerous than elsewhere in the plant? Can you give any reasons for the presence of this band of cells in this particular portion of the thallus? Are any of these cells apparently branched?

Examine the nearly colorless cells making up the greater part of the section. What is their shape? Do you find them so arranged as to leave many intercellular spaces? What do these cells contain? What portion of the midrib do they form? Do any of these cells show pit-like markings in their walls?

- b. The **rhizoids**.—With the fine forceps tear off a bunch of rhizoids. How many kinds do you find? How do you distinguish them? What is their structure? Examine your sections for rhizoids. From what cells in the thallus do they grow? What do the rhizoids contain?

- c. The "**leaves.**"—Note also the sections of the "leaves" among the rhizoids. What is the structure of these bodies?

Draw portions of your sections to show the structures studied.

Make longitudinal sections through the thallus and compare them with the sections just studied. Draw.

- d. The **stomata**.—Select a flat thallus, lay a portion of it on a slide, and examine with a high power. Note the distribution of the stomata. Select a single well-formed stoma and study its structure. What is the shape of the stoma? Of how many cells is its rim formed? What is their shape? How are they arranged? Do they contain chlorophyll? Focus down into the cavity of the stoma and note the **guard cells**, which project into and partly close the opening of the stoma. Examine the under surface of the thallus for stomata. From the same or another flat thallus make tangential sections, *i. e.*, sections parallel to the flat surface of the thallus, so as to remove only the "epidermis." Examine these with the high power and compare with the other preparations which show the structure of stomata. Parts of the section will probably show the manner in which the green cells lying immediately under the "epidermis" are divided into groups.

Draw a sectional and a surface view of a stoma.

- e. *The fruiting organs.*

1. The **cupules** and **gemmae**.—With a dissecting-needle pick out of the cupules some of the gemmæ, mount them in a drop of water, and examine under a low, then a high power. What

is their shape? Of what kind of tissue are they composed? Are they of the same thickness throughout? Do the cells have the same contents as those of the thallus? Examine the margin of a single gemma and find the **scar** at the point where the gemma was attached to its **pedicel** or **stalk**. What is the shape of the scar? What is the arrangement of the neighboring cells? Find also two **vegetative notches**. How are they situated in reference to each other and to the scar? How do you distinguish them from the scar? What is the position of the margins of the gemma on each side of the notch? Examine the base of the notch in large, mature gemmæ for small papillæ, which are the early stages of the young plants to which the gemmæ give rise. Why should these organs be called "gemmæ"? Having examined one surface of a gemma, turn it over and examine the other side. Are the two sides unlike? In what ways may gemmæ be distributed? What position would they naturally assume upon the ground? Is their structure at all related to the manner in which they produce new thalli?

Draw several gemmæ of various sizes. Cut off a cupule and mount it in a drop of water. Examine it with a low power. Notice again the shape of its margin. Put on a high power and study the structure of the cupule, noting the gemmæ on the inside, and its toothed margin. Draw. Examine a young cupule. How does it differ in structure from the mature organ? Draw. Make transverse sections through

a thallus, passing through the cupule, and mount in fifty per cent. glycerine. Study the wall of the cupule. What is its structure? Of how many layers of cells does it consist? Note the base. Examine the gemmæ inside the cupule. To what part of it are they attached? How are they attached? In what direction do they stand? What is the structure of the pedicel? Look for gemmæ of various sizes. Can you trace them down to two-celled papillæ? Study a number of sections to see that a gemma develops from the division of the upper cell of a papilla, while the lower cell remains as the pedicel. Do you find any unicellular papillæ? Draw.

2. **The antheridial branch.** — Make transverse sections of the stalk. What is its outline? What is the position of the grooves? Of what sort of tissue is the stalk composed? Does it have an epidermis? Do its cells contain chlorophyll? What is the arrangement of the margins of the groove? Note in the grooves the rhizoids, seen in cross-sections. Are there many or few? What is their outline? Note the peg-shaped outgrowths projecting into their cavities. Compare this view with a longitudinal view of a rhizoid. Notice also the sections of scales in the groove. Do you find them elsewhere in the section? Compare with those seen on the under side of the thallus. Draw the section. Select a large, well-developed antheridial branch, and make a vertical section which passes down through the middle of the receptacle and stalk. Mount in water or gly-

erine and examine under the low power. What is the shape of the section? Note in the receptacle flask-shaped, dark-colored masses, the **antheridia**. Of what tissues is the receptacle composed? Compare with the thallus. Do you find scales, rhizoids, etc., on the section? If so, to what part are they confined? Examine under the high power. Note that each antheridium is contained in a well-defined cavity. What is the shape of an antheridium? Does it have a stalk? What is the structure of the wall of the antheridium? Do the cells contain chlorophyll? How are the cells arranged? Can you make out its contents? Do you find unicellular papillæ, the **paraphyses**, at the base of the antheridium? How does the antheridium communicate with the exterior? Do the antheridia vary in size and development in different parts of your section? If so, where are the oldest? Draw the section. If living material containing mature antheridia be used, some of the sections will probably show escaping **antherozoids**. If so, study their structure and movements. Compare them with the antherozoids of *Chara* and *Fucus*. Draw.

Make a horizontal section of the surface of the antheridial receptacle, and examine under the high power. Do you find that it has the same structure as the surface of the thallus? Note the smaller openings or **pores**, leading to the antheridial cavities. Do these pores differ in structure from the stomata? Draw.

How many points of resemblance in structure can you discover between an antheridial branch and a thallus?

3. The **archegonial branch**.—Make sections of the archegonial, and compare them with the sections of the antheridial branch. Do you find any marked differences in shape, size, structure, etc.? Do you find the same tissues to be present and similarly arranged? Draw the sections. Remove one of the arms of the archegonial receptacle, make transverse sections of it, and compare the structure with that of the stalk. Examine vertical sections, and find on the lower surface of the receptacle the flask-shaped **archegonia** with elongated necks. Notice how these are distributed through the section. Do you find them to be arranged in pairs, with a wing-like down-growth (sections of the **perichæstium**) on each side? Draw.

Study the structure of a single archegonium which has been fertilized. These may usually be distinguished from others by the fact that the lower portion contains a dark-brown mass. Make out the **stalk**, the **body**, and the elongated **neck**. What is the structure of the stalk? From what does it arise? Does it resemble the stalk of an antheridium? Surrounding the stalk look for the section of a cup-shaped mass of cells, the **perigynium**. How much of the archegonium does the perigynium cover? What is its structure? What is the shape of the body of the archegonium? What is the structure of its wall? What does it contain? Do the contents entirely fill the cavity? Can you



make out the structure of the contents? How is the neck formed? How does it compare in length and diameter with the body? Draw a single mature archegonium. Examine the section for younger, unfertilized archegonia. Where do you find them? Does their position correspond with that of the immature antheridia? Draw several young archegonia to show the various stages of their development. Compare the young with the older archegonia. Look on older receptacles for the **ripening archegonia**, or **sporogonia**, and study their structure. What changes have taken place in the perigynium, in the neck, and in the body? Draw. Crush a living sporogonium and examine the **spores** and spirally-marked **elaters**. Mount them without the drop of water and examine, then allow a small drop to flow under the cover, being careful to keep watch of the elaters. What change takes place in them when they are moistened? Of what use may this be? Draw some of the spores and elaters.

#### PHYSIOLOGY

*a. The fertilization of the öosphere.*

If antheridia and archegonia of the proper age be obtained, it will not be difficult to watch certain stages of the process of fertilization. Make vertical sections of the two receptacles, bearing respectively mature antheridia and archegonia, and mount them in the same drop of water. Note the motions of the antherozoids, their jour-

ney to the necks of the archegonia, and their behavior after reaching the latter. Can you give any reason why the antheridia are on the upper, and the archegonia on the under side of the receptacle?

### **Fern (*Aspidium Sp.* or *Pteris Sp.*)**

*Material.*—Ferns are almost everywhere abundant in woods, meadows, and along the roadside, edges of fields, etc. Some specimens should be taken in the early spring as the fronds are coming out of the soil, others in the early summer when the vegetative fronds are mature, and still others later in the summer and in the early fall, when the spores are to be found in different stages of formation. Prothallia are to be looked for on mossy logs and rocks and on the soil at the base of the fern plant. Some of the specimens should be pressed as for an herbarium, and others preserved in alcohol. Species closely related to, or even identical with, our wild forms are to be had at almost all greenhouses, and are available in midwinter. Their prothallia are usually abundant on the soil of the neighboring flower-pots and beds, or adhering to the surface of the flower-pots themselves, particularly of those which are covered with a film of vegetable growths. Wild ferns may be kept in a greenhouse if wanted for examination in winter. Their prothallia may be raised from spores strewn upon the surface of clean, damp sand. The cultures should be examined every few days, and the different stages of development studied as they become available. The young fern plants will develop from the prothallia in about six or eight weeks. The prothallia may be picked off the surface of the sand with needles and examined in a drop of water. They may be preserved by being

placed in a saturated solution of picric acid for six or eight hours, washed for a few minutes in thirty per cent. alcohol, and then hardened in the various grades of alcohol. In addition to the plants the student will need the compound microscope, hand-lens, forceps, razor, dissecting-needles, watch-glass, scalpel, ten per cent. hydrochloric acid, dilute glycerine, Schulze's solution, Schulze's macerating mixture, acetic acid carmine, hæmatoxylin, picric acid, alcohol lamp, pith, and fifty per cent. alcohol.

*Method of Examination.*—Living plants should first be studied in their various relations to their surroundings. Dried specimens are good for the study of the gross anatomy, especially of the frond. Alcoholic material should be examined in fifty per cent. alcohol. The rhizome and fronds should be sectioned in various directions, and the disposition of the different tissues studied. Sections for microscopic examination are also needed. Material preserved in strong alcohol must be soaked one to four hours in seventy-five per cent. alcohol before sections are cut.

#### MORPHOLOGY

##### *A.—The Spore-bearing or Asexual Plant.*

##### *Naked-eye Characters.*

- a. General appearance.*—Study a well-developed specimen as it grows in the soil, and note that the aerial portion consists mainly of long-stalked leaves, the **fronds**. On their upper portions the stem or **rachis** of each frond bears lateral outgrowths or appendages, the **pinnæ**, resembling leaflets. Each **pinna** is subdivided into smaller portions, the **pinnules**. On the back of the pin-

nules are found circular brown dots, the **sori**, each of which, on being examined with a lens, is seen to consist of a membranous expansion or **indusium**, covering a group of small stalked bodies, the **sporangia**, in which on microscopical examination are found the **spores**. Among the bases of the fronds of the current year will be found many dried stalks, the remains of leaves of previous years. Upon removing the plant from the soil and washing it in water it will be seen that the leaves arise from a stem-like portion or **rhizome**, which creeps along just below the surface of the soil and gives off roots.

b. *Structure*.—Remove a plant from the soil, wash the roots, and study the following parts :

1. The **rhizome**.—What is its general shape? Does it branch? Cut the rhizome across, midway between its ends. What is the shape of the outline? From what part of the rhizome do the roots arise? From what part the fronds? Are these positions constant? Do you find any other structures than roots and fronds borne by the rhizome? Does the rhizome appear to be made up of segments, as **nodes** and **internodes**? If so, how do you distinguish the nodes? On what part of the rhizome are the leaves of the current year borne? The remains of leaves of past years? What is the general structure of that end of the rhizome which is near the leaves of the current year? What is the color of the margin of the rhizome, as seen in the cross-section? Of the greater portion of its centre? Do you find near the centre any

parts which are of a different color from the surrounding tissue? From the margin? If so, how many of each kind are there, and how are they arranged?

Make a drawing of the cross-section, showing its shape and the position of the various tissues.

Divide a portion of the rhizome through the middle lengthwise. How do you find its tissues to be arranged? Look for strands of fibrous tissue. In what direction do they run? With a fine scalpel carefully remove the tissue surrounding these strands. What is their arrangement in the rhizome? Do they enter the leaves and roots? These strands may be more easily traced if the rhizome be soaked for a few hours in ten per cent. hydrochloric acid. This macerates the softer tissues, which may then be picked away from the fibrous bundles.

Make a drawing showing the arrangement of these bundles.

2. The roots.—Are they numerous or few? What is their color? Are they rigid or flexible? Do they interlace in a mass or spread out? Do they branch? If so, are the branches given off in definite order? What is the average length of a root? Diameter? Cut a large root in two and examine the cut end. Is the root made up of tissues visible without using a microscope? If so, how do you distinguish them? Is the surface of the roots smooth? Examine one of the smallest roots with a lens. Notice the fine **root hairs**. Do they grow at the extreme end of the root? If not, how far from the end do they stop? Note the white growing end.

How long is it? At the tip look for the brownish **root-cap**. How does it compare in length with the growing end? Judging from the facts just learned, what do you consider to be the principal differences between a root and a rhizome?

Make a drawing of a single large root.

3. The **fronds**.—How many does your specimen bear? To what extent do they vary in length? Lay an entire frond on a flat surface. What is the shape of the outline? What is the shape of the rachis? Color? Size? Is its surface rough or smooth? Does the rachis branch? How many pinnæ does it bear? Is the number constant? What is their position with regard to one another? How far above the surface of the soil is the first pinna borne? What is the shape of a single pinna? Size? Is its surface smooth? Compare with the rachis. What variations in shape and size do you find? How are the pinnules arranged? Do they vary at all in number, shape, and size on different pinnæ? Note the **midribs** of the pinnæ. Do the pinnules also have midribs? If so, what is their relation to those of the pinnæ? On which side of the pinnules are the sori borne? Is this an invariable position? Do they have a definite arrangement? If so, what is it? Does each pinnule have a constant number of sori? Examine a number of fronds to find sori in various stages of growth. What is the first indication that a **sorus** is to be formed at a particular place? What changes take place in it during its development? What is the shape

of the indusium? Size? How is it attached? What changes take place in it as it develops? Draw a single pinna, showing both surfaces.

Look for young fronds and notice the manner (*circinate*) in which they are rolled up in the bud.

*Microscopic Structure.*

- a. The **rhizome**.—Cut a series of transverse sections of the rhizome, mount the thinnest and most perfect in a drop of water or dilute glycerine, and examine under the low power. Make out the following parts: on the surface a single layer of cells, the **epidermis**; within this a band of dark-brown cells, **sclerenchyma**; enclosed by the band of sclerenchyma, a ground mass of light-colored cells, **parenchyma**; embedded in the parenchyma, isolated groups of sclerenchymatous tissue and large masses of yellowish tissue, the **fibro-vascular bundles**.

Make a diagram showing the position of all these parts. Put on the high power and study each of these in detail.

1. The **epidermis**.—How many cells in thickness is it? What is the shape of the cells? What do they contain? How do the different walls of an individual epidermal cell compare in thickness? Examine sections in which the margin is perfect, and note whether or not the epidermis is always present. Does the epidermis present any outgrowths? If so, what are they? Is the epidermis broken at places to allow of the passage of outgrowths from underlying tissues? Draw a portion of the epidermis.



2. The **sclerenchyma**.—How do you distinguish it from the epidermis? What is the color of its cell-walls? What are its cell contents? Are the walls stratified, pitted, or marked in any way? Note the line (section of the **middle lamella**) between the adjacent cell-walls. Are there any intercellular contents? Does the *peripheral sclerenchyma* pass entirely around the stem? Examine the groups of *central sclerenchyma*. How many are there? Of what are they composed? Are the component parts different from those of the peripheral sclerenchyma? Draw some of the sclerenchyma.
3. The **parenchyma**.—Is this sharply marked off from the sclerenchyma? How do its cells compare in shape, size, color, and contents with those of the sclerenchyma? Compare them with the parenchyma cells seen in *Marchantia*. Make drawings of the parenchyma.
4. The **fibro-vascular bundles**.—Are they visible to the unaided eye? How many do you find? Are they arranged in definite positions? What is their shape? Are they sharply separated from the surrounding tissues? Note that each bundle consists of a group of large, thick-walled, empty cells, the **xylem** portion of the bundle, surrounded by a band, the **phloëm** portion, of smaller, thinner-walled cells, with granular contents. Outside of this is a narrow band, the **bundle-sheath**, of cells, next which comes the parenchyma. As sections of the rachis are much more easily cut than of the rhizome, the detailed examination of the fibro-vascular bundle will be made when the leaf is studied. Stain

a section with Schulze's solution, and note especially the color assumed by the cell-walls and cell contents of the different tissues. The cell-walls which stain yellow are lignified. Stain another section with acetic acid carmine, and a third with an aqueous solution of hæmatoxylin. Compare results. Make longitudinal sections. Under the low power note especially the course of the fibro-vascular bundles where they branch to enter the appendages of the rhizome; also the course of the strands of central sclerenchyma. Examine under the high power, and note again the shape of the cells of the various tissues. Examine in the vascular bundles the long, thick-walled elements, the **scalariform tracheides**, marked with narrow parallel pits. Stain these sections as above and compare with the transverse sections. Draw.

Carefully remove the bases of the fronds covering the extreme apex of the rhizome, and with a lens examine the extreme papilla-like end, the **apical cone** or **growing point**. Make longitudinal and transverse sections of the growing point of two or more rhizomes, and look for the triangular **apical cells**. Study the relation of the apical cell to the surrounding cells.

Cut thick sections of the rhizome, mount them on a slide in a few drops of Schulze's macerating mixture, and warm the slide over an alcohol lamp. After a few minutes the section will be resolved into its constituent cells, which may then be studied separately. Note particularly the shape and markings of the various elements. Draw.

- b. **The root.**—Make transverse sections of a large root and examine with the low power. What is the outline of the section? What is its color? Do you find the tissues arranged in the same manner as in the rhizome? If not, what are the differences? How many vascular bundles are present? Notice the **root-hairs**. Examine with the high power. What tissues are lacking? Do you find that the vascular bundles of the roots have the same general structure as those of the rhizome? Have they the same shape? Make a diagram of the arrangement of the tissues as seen in this section. What is the shape of the root-hairs? What is their color? From what tissue do they grow? What is their structure? Contents? Compare those found on a large root with those near the tip of one of the smallest rootlets. What differences do you discover? Draw several root-hairs. Examine a section showing a surface view of the epidermis, and note the shape and arrangement of the cells and of the root-hairs. Draw.

Study longitudinal sections and compare them with the transverse and with longitudinal sections of the rhizome. Note particularly the arrangement of tissues at the places where rootlets are given off. Draw.

Cut off the tip of a young root about a half-inch from the end, hold it between two pieces of pith, and cut it into very thin longitudinal sections. Examine these under the low power for the section which passes through the middle of the root and contains the **apical cell**, which lies a short distance back from the extreme tip of the root. Mount this section in a drop of dilute gly-

erine and study under the high power. Note that the tip of the root is covered by a mass of cells forming the **root-cap**. What is its shape as seen in section? Of what kind of cells is it composed? What is the shape of the apical cell? How do you distinguish it from the surrounding cells? How many cells is it from the tip of the growing portion? Note the **segmental cells**. Stain with acetic acid carmine. Try to see that some of the segmental cells go to form the growing portion of the root-cap, while others are added to the root itself. From what part of the apical cell does each arise? Study the arrangement of the tissue, the **meristem**, around the apical cell, and note the *differentiation* that is gradually taking place. Make drawings to illustrate the structure of the tip of the root. Make transverse sections of the root-tip and compare. Draw. Compare the structure of the root-tip with that of the apex of the rhizome.

c. The **fronds**.

1. The **rachis**.—Make transverse and longitudinal sections of the rachis midway between the lowest pinnæ and the ground, and examine under the low power. What is the shape of the section? What is its color? Is the arrangement of the parts like that in the rhizome or that in the root? Note the **trichomes** or hairs around the margin of the section. Put on the high power. Do you find the same tissues present as in the rhizome? What parts of the section contain chlorophyll? Study the epidermis and compare with that of the rhizome. Do the trichomes originate in the same manner as the root-hairs?

Do they resemble the latter in structure? Is the sclerenchyma arranged as in the rhizome? Do the sclerenchymatous elements have the same shape, size, contents, etc., as in the latter? Compare the parenchyma in the two parts. Study carefully the **fibro-vascular bundle**. Note the position and shape. Study the **bundle-sheath**. Of what kind of cells is it composed? Of how many layers of cells is it formed? What is the structure of their walls? What do the cells contain? Do you find intercellular spaces present? Within the bundle-sheath find the **phloëm-sheath**, consisting of parenchymatous cells containing starch. What is the shape of this sheath? Does it everywhere have the same thickness? Do the cells contain protoplasm? Inside the phloëm-sheath comes the **bast** or **phloëm**, the outer portion of which consists of thick-walled elements, the **protophloëm**. What is the shape of the cavities in this layer? Are there any intercellular spaces? Next within this comes the true **phloëm**, consisting partly of elongated, thin-walled elements, the **sieve-tubes**, and partly of parenchymatous cells, the **bast parenchyma**. How many rows of sieve-tubes are there? What is their shape in cross-section? What do they contain? What is their position in regard to the bast parenchyma? The centre of the bundle is occupied by the **xylem** or **wood**, consisting mainly of thick-walled elements. Note the polygonal shape of some of the xylem elements, the **tracheids**, when seen in cross-section. How are these elements arranged with regard to one another? What do

they contain? In longitudinal section they appear as the scalariform tracheides already noticed. Among the elements of the xylem look for parenchymatous cells, the **wood parenchyma**. How do you recognize them? What is their position? What do they contain? How do they differ from the bast parenchyma? Draw a fibro-vascular bundle as seen in cross and in longitudinal section.

Stain the sections with Schulze's solution, and note carefully the color assumed by the various elements of the bundle.

Examine sections of the rachis made at points where pinnæ are given off and others made near the upper end of the frond, and compare with those from the lower portion. Note the differences in the sections and the changes due to age. Study also sections made near the junction of the rachis with the rhizome.

Draw the various structures observed in all of these sections.

2. The **pinna**.—Mount a piece of one of the pinnules which bears no sori, and examine both surfaces under the low power. What is the nature of the surfaces? Are both alike? Of the margin? Do you find any **veins** or fibro-vascular bundles which are not visible to the naked eye? Does the green tissue, the **mesophyll**, extend to the extreme margin of the pinnule? Cut horizontal sections parallel to the surface on each side of the pinnule, thus removing the epidermis, and examine with the high power. What is the shape, as seen in surface view, of the epidermal cells covering the mesophyll?

Of those covering the veins? Study the trichomes. What is their shape? Structure? Do they originate from particular cells? How do they compare with the root-hairs and with those found on the rachis? Look for **stomata** surrounded by two kidney-shaped **guard-cells**. Do you find them on both surfaces? Are they abundant or few? What do the guard-cells contain? Do the epidermal cells have the same contents? Compare them with those seen in *Marchantia*.

Make drawings showing the arrangement of the cells of the epidermis. Study transverse and longitudinal sections of the pinnule. Does it vary in thickness? Note the structure and arrangement of the epidermal cells. Do they contain chlorophyll? How many layers in thickness is the mesophyll? Notice the arrangement of the fibro-vascular bundles. Draw a cross-section as seen under the high power. Mount without the drop of water and cover-glass a portion of a pinnule bearing mature (brown) sori, and with the low power examine its under surface. Note the **indusium** covering dark-brown or black bodies, **sporangia**. What is the shape of the indusium? Color? Does it entirely cover the sporangia? Does it bear trichomes? Does it differ in this respect from the epidermis? With a dissecting-needle remove an indusium to the slide and mount in a drop of fifty per cent. alcohol. Expel the air, then examine under a high power. What is the structure of the indusium? Do its cells resemble those of the epidermis? Does it have stomata? In the same

manner study young sori. Examine some of the mature sporangia in a drop of water. Notice that each consists of a **stalk**, which bears the **capsule**, within which are the **spores**. What is the structure of the stalk? Of how many rows of cells does it consist? What is the structure of their walls? What do they contain? What is the shape of the capsule? Note the marginal cells forming the **annulus**. What is their shape? Color? Notice particularly their arrangement and the structure of their walls. Look for sporangia which have broken open along the **line of dehiscence**. Where is this line? If no such sporangia are found, crush some by pressing on the cover-glass, or run a drop of glycerine under the cover-glass. Draw closed and open sporangia.

How many spores does a sporangium contain? How are they arranged? Examine a single spore and note its shape, size, color, structure, contents, etc. Draw several spores. Examine sections made through pinnules bearing mature and those bearing young sporangia. Endeavor to make out how the sporangia are attached in the sorus, how they develop, and how the spores are formed within the capsule. Are all the sporangia in one sorus of the same age? Can you trace any structural relationship between the sporangia and the trichomes? Note also the structure of the indusium.

Make drawings of each structure examined.

*B.—The Prothallium or Sexual Generation.*

Procure prothallia from the greenhouse, or



raise them in the manner to be described hereafter. Place a young prothallium in a drop of water and examine under the low power. What is the shape of the prothallium? Color? Look for **rhizoids** on the lower surface. Are they confined to any particular region? Draw. Examine with the high power. What are the general characters of the cells composing the prothallium? What do they contain? Look for the **growing point** at the base of the depression in the margin. How do the rhizoids differ from the root-hairs on the fern-plant? From the rhizoids of *Marchantia*? Among the rhizoids look for knob-like bodies, the **antheridia** and the **archegonia**. The former may be distinguished in surface view as consisting of a small circular eminence of a single large cell, or of several small ones without any opening between them; each archegonium usually appears as a ring of four cells surrounding a plainly visible opening, the mouth of the **canal**. What other differences can you distinguish between these two organs? In case mature specimens are examined, many **antherozoids** will probably be found swimming around in the water. If so, study their movements and structure. Note the **body** of the antherozoid and the **cilia**. To what part of the body are the latter attached? Are they numerous or few? Look for the **vesicle**, which is usually attached to the body. Draw several antherozoids. To make out the structure of the archegonium, sections of the prothallium should be cut. Successful sections will show an archegonium to consist of a **neck**—down which runs a **canal**, whose **mouth** was seen in the surface view—

and a **body**, in which may be distinguished an **oosphere**. Study the structure of these parts and compare with the corresponding parts of *Marchantia*. Draw.

#### PHYSIOLOGY

*a. The germination of the spore and the development of the prothallium.*

Sow a few spores in a drop of water in a moist chamber. How long before the spore ruptures? What changes take place in it at this time? What is the shape of the embryo prothallium? How long before the primary root-hairs develop? Only the first stages can be observed in water cultures. To get the later developmental stages, spores may be sown on slabs of plaster of Paris, pieces of porous earthenware, or, best of all, on clean, damp sand. The cultures must be kept damp and be examined every few days. On older prothallia, look for the first rudiments of the asexual generation (the fern-plant proper), which in sections will be seen issuing from an archegonium which has been fertilized. Still later stages will show a single leaf borne above the prothallium, and from this point the development may be watched with the naked eye.

## **The Flowering Plant**

### **A.—SEEDS**

*Material.*—Select a number of well-formed dry seeds, including beans, peas, corn, cucumber, watermelon, oak, maple, flax, and mustard.

Other material and apparatus required: an egg, corn-starch, iodine, barium hydrate, sodium hydrate, one per cent. solution of copper sulphate, grape-sugar, distilled water, Millon's reagent, diastase of malt, a few test-tubes, parchment, gauze, blotting-paper, filter-paper, bell-jar, tumbler, razor, cork, scalpel, small funnel, alcohol lamp, forceps, dividers, metric scale, hand-lens, chemical thermometer, compound microscope, dialyzers, and a piece of glass tubing four inches long and two inches in diameter.

### **MORPHOLOGY**

Soak a number of large seeds—*e. g.*, beans, peas, corn, etc.—in water overnight in order to soften them.

I.—*Bean*, notice :

*a. Shape.*—What is the shape of the bean as viewed from the side? From the edge? From the end? Compare several different kinds to see the variations in shape. How do you account for the shape?

- b. Size.*—What is the length? Breadth? Thickness? Measure several carefully. What variations in size?
- c. Color.*—What is it? What variations? Is the color in the covering or in the fleshy portion? Of what use is the color? What is the cause of it?
- d. Structure.*
1. The **seed-coat** or **testa**.—What is its structure? Color? Use?
  2. The **hilum**, or scar left by the seed-stalk or funiculus.—What is its position? Shape? Size? Use?  
Near the hilum find
  3. The **micropyle**.—Examine several seeds to see if its position is constant. What is its shape? Size? Use?  
Tear away the testa and note
  4. The **cotyledons**.—How many are there? What is their position? Shape? Color? What relations do they bear to the embryo as regards position and size?
  5. The **embryo**, consisting of **caulicle** and **plumule**.—What is its shape? Is it attached to the cotyledons? If so, how? How do you distinguish the caulicle from the plumule? In what direction does the tip of the caulicle point? Examine several beans to see if it always points in this direction. What is the position of the tip of the caulicle with relation to the micropyle? What is the structure of the caulicle? Shape? Size? What is the position of the plumule? Of what is it composed? Compare

it in shape, size, and structure with the caulicle.

Make sketches of the bean, illustrating all of these structures.

II.—Examine in like manner seeds of the watermelon, cucumber, pea, oak, maple, and corn. Compare them with the bean in all respects and explain the differences.

Draw each kind of seed examined to show its structure.

What are the structural differences between a seed and a spore?

III.—*Some of the Chemical Contents of Dry Seeds.*

*a. Tests for starch.*

1. Fill a test-tube one-third full of water, add a pinch of dry corn-starch, and shake the tube. Does the starch dissolve? Heat the tube to the boiling-point over the flame of an alcohol lamp, keeping the tube agitated during the process to keep the starch from settling to the bottom of the tube. What change takes place? Explain. Set the tube aside to let the starch cool.
2. Fill another tube one-third full of water, and add a drop of iodine. What result? Explain.
3. Add a drop of iodine to the cold starch in the first test-tube and shake the tube. What result? Compare with 2. To what is the difference in results due?
4. Peel the testa from several dry beans, break the beans into small pieces, place the pieces in a test-tube containing water, and boil them for several minutes. Then cool the tube and di-

vide the contents into two parts. Test the first part with a drop of iodine. Does the bean contain starch? How do you tell? Filter the second part until perfectly clear, and test the filtered fluid with iodine. Is starch present? Does starch dissolve when boiled?

5. Test the testa for starch. Does it contain any starch? In what part of the seed is starch stored? Put a drop of iodine on the surface of an uninjured cotyledon of a soaked bean. What result? Explain. Cut the end off the cotyledon, and apply a drop of iodine to the cut surface. What result? In what part of the cotyledon is the starch stored?

*b. Tests for grape-sugar.*

1. Put some grape-sugar (glucose) into water in a test-tube. Does the sugar dissolve? Do you think that if a plant-cell contained grape-sugar it would be dissolved in the watery fluid (*cell sap*) forming the vacuole? Compare with the starch. Test the sugar with iodine. Do you get the same reaction as with starch? Why?
2. Fill a test-tube one-fourth full of water, add an equal amount of sodium hydrate, and shake the tube. What change? Add two or three drops of a one per cent. solution of copper sulphate. What change? Shake the tube vigorously. What change? Boil the contents of the tube for a minute or two. What change?
3. Into a test-tube one-fourth full of water put a pinch of grape-sugar, then add the sodium hydrate and copper sulphate as before, and shake the tube. Then boil for a minute or two. Do

you get the same result as you did without the grape-sugar? To what, then, is due the change noticed in this last experiment?

4. Repeat the preceding experiment, using starch instead of grape-sugar. Do you get the same result as with grape-sugar? Why? Can you, then, by using sodium hydrate and copper sulphate, detect the presence of grape-sugar in a solution? Could you detect it by merely looking at the solution, *i. e.*, without the use of chemicals?
5. Prepare some dry beans or corn as previously directed. Filter the contents of the test-tube used into another tube, add sodium hydrate and a few drops of copper sulphate, and boil. What result? Is grape-sugar present in the dry seed? How do you tell?

*c. Tests for albumen (aleurone).*

1. Into a test-tube about one-third full of water put two or three drops of the white of an egg, and shake the tube. Pour into the tube ten or twelve drops of sodium hydrate. Shake again, add one or two drops of a one per cent. solution of copper sulphate, shake vigorously to mix thoroughly the contents of the tube, then boil. If the deep-blue color formed by the mixture of the two reagents changes to a purple, either before or after boiling, the presence of an albuminous substance is indicated.
2. Repeat the above, using boiled starch in place of the white of egg. Do you get the purple color? Why?
3. Prepare some beans free from the testa, as was

done for the starch tests, boil the pieces in water for a few minutes, then pour into the test-tube ten or twelve drops of sodium hydrate and one or two of the copper sulphate, and boil again. Does the color indicate the presence of an albuminous substance?

4. Repeat the last experiment, using a few drops of Millon's reagent in place of the other two chemicals. If the contents of the test-tube turn pink or red the presence of an albuminous substance is indicated.

#### IV.—*Microscopic Examination of Starch and Aleurone.*

Mix a pinch of starch and some water together in a watch-glass, then put a drop of the mixture under the microscope, and note the shape, size, color, and structure of the starch grains. Carefully run a drop of iodine under the cover-glass, and note the effect on the starch. With a sharp razor cut a very thin section across a softened cotyledon of a bean, examine the section under the microscope, and note how the starch is stored away in the cells. Does this give you any clue to the explanation of the results obtained in 5 under "Tests for starch"? Test the section with iodine. Note the very small aleurone grains in the starch-bearing cells. What color do they assume when stained with iodine? Note the aleurone grains stored in the cells near the surface of the cotyledon. Draw.

Focus upon the surface of a cell-wall, and notice the small circular openings, the **ends of canals**, which perforate the wall. Focus upon the edge of a wall between two cells, and note



the length of these canals. Compare the diameter of the canals with that of a starch grain. Are the canals large enough to admit of a starch grain passing through them from one cell into the next? Can, then, the starch grains be carried into and out of the cells by the flow of sap through the tissues of the cotyledon?

Compare the results of all your experiments on the dry seeds. Do you find starch present? Grape-sugar? Aleurone? In what form does each of these exist in the seed?

#### PHYSIOLOGY

##### *a. Imbibition and turgescence.*

1. With a pair of dividers measure the length, width, and thickness of several dry beans, then put them into a dish of water, and examine at intervals of three to five minutes. Explain the changes which take place. Let the beans remain in the water overnight. What change? Explain. Compare the measurements of the soaked with those of the dry beans. Is the average increase in the size of the beans due to the imbibition of water? Let the beans dry in the air. Do they regain their original size?
2. Pack a thin glass bottle full of dry beans, pour in enough water to cover them, fasten the stopper tightly with a cord or wire, set the bottle aside for a few hours, then examine. Explain the result. Do seeds imbibe water in spite of great external pressure? Compare the behavior of the seeds in this experiment with that of a

sponge immersed in water, but held tightly squeezed in the hand.

3. Provide a glass tube about four inches long and two inches in diameter and open at each end, two pieces of thin parchment well soaked in water and sufficiently large to tie over the ends of the tube, about two table-spoonfuls of syrup (or, better, the same amount of grape-sugar or glucose), a piece of strong twine, and a dish holding about a pint of distilled water. Over one end of the tube tie a piece of the moistened parchment so tightly that it cannot slip off, but leave that part of it which covers the calibre of the tube somewhat loosely wrinkled or plaited. Pour in the syrup, diluted with sufficient water to fill the tube, or put in the dry grape-sugar, and fill the tube with water. Tie over the open end the second piece of moistened parchment, arranged like the first. Turn the tube first on one end, then on the other, to see that none of the syrupy fluid within can come out, then place it in the dish of distilled water. Examine the tube from time to time to see whether or not the parchment is become tense and outwardly convex. What makes the parchment bulge? Are the conditions of the experiment at all similar to those of a plant-cell—*e. g.*, one of the cells of *Spirogyra*—immersed in water? Compare the end walls of the tube with the ends of a filament of *Spirogyra*. If a plant-cell whose walls were more or less flaccid, and whose contents were a fluid—*i. e.*, cell-sap—denser than water, be placed in the latter, would the cell absorb some of it? Would the walls become

tense and stretched, *i. e.*, turgid? What changes would take place in a tissue composed of such cells?

*b. Germination.*

Sow some beans, corn, and pumpkin seeds in damp sawdust or sand, and put some flax and mustard seeds on damp blotting-paper under a bell-jar. Examine from day to day, and note the changes which take place. What is the first visible sign that germination has begun? On which day does the radicle appear? On which the cotyledons and caulicle? What becomes of the seed-coats? Note the behavior of the cotyledons—how they are withdrawn from the seed-coats and from the soil, how they expand or unfold, and the changes which take place in the shape, size, and color of the cotyledons as growth goes on. When do the first true leaves unfold? When does the second pair appear? In which direction does the primary root grow? Examine the roots for root-hairs. Where do they first appear? Do they grow at the extreme tip of the root? Examine with a microscope the hairs on a root which is two or three inches long. How do the hairs on the lower differ from those on the upper end? How do you explain the difference? Sow some mustard seeds in loose, damp sand. After they have germinated, carefully draw them out of the sand along with the particles attached to the root-hairs. Wash gently in water to get rid of the unattached particles. Do you find that much of the sand adheres to the root-hairs? What do you consider to be the function of these

hairs? Compare with those seen on the fern roots.

Allow some seeds to germinate in the light, others of the same kind in the dark. Explain the differences.

Look under the trees for germinating acorns, horse-chestnuts, maple, and pine seeds, and endeavor to explain the various peculiarities found.

Do the cotyledons of any of the seeds contain chlorophyll before germination? After? Do those with chlorophyll have thick or thin cotyledons? Can you give reasons?

Fill a tumbler about half full of water, tie loosely over the mouth of the tumbler a piece of gauze, allowing it to hang down into the tumbler, but not to come in contact with the water. On the gauze place a few flax seeds, grains of wheat, oats, etc., cover the whole with a bell-jar, and set in a warm place near a window. Do the seeds germinate? If so, do they germinate as quickly as those placed on blotting-paper or damp sand? Whence do they get the moisture needed for germination? Judging from this experiment, would you regard seeds as having a very strong tendency to absorb moisture from the soil, although there might be but little moisture present? How does this agree with facts observed in the garden and in the field?

*c. Geotropism of seedlings.*

Pin a germinating bean or kernel of corn to a flat piece of cork, with the bean upon the upper surface, and with the radicle pointing upward and the caulicle downward. Float the cork in some

water in a dish; cover the dish with a bell-jar or tumbler in order to prevent evaporation, and study the behavior of the radicle and caulicle. Explain.

Make drawings illustrating the facts observed.

*d. Respiration.*

Allow some beans or peas to germinate in a corked bottle with a wide mouth. When they are well started, pour some barium hydrate into a watch-glass, invert the bottle over the glass, and carefully withdraw the cork, holding the mouth of the bottle close to the surface of the liquid. What change in the liquid? Explain.

*e. Temperature of germinating seeds.*

Put some germinating seeds into a beaker under a bell-jar having an open top. Close the top with a stopper having two openings, into each of which is thrust a chemical thermometer. Push one thermometer down until its bulb is buried among the seeds. After a few hours take the readings of the thermometers. Explain.

*f. Some of the chemical contents of germinating seeds.*

Take a number of germinating seeds, *e. g.*, corn, which are just beginning to develop roots and stems, with a knife cut the seeds into small pieces, boil them for a few minutes in a test-tube, and divide the contents of the tube into three parts. Test one part with iodine for starch. Do you find it present? Test the second part with sodium hydrate and copper sulphate for glucose. Is it present? Test the third portion for the presence of proteids with Millon's reagent. Do you find

any proteid substances? Compare the chemical contents of germinating with those of dry seeds. Do you find anything present in the germinating which is not in the dry seed? Bake some dry seeds to destroy their power of germination, then place them in water. Do they still absorb water? If so, test some of them for starch and others for glucose. Is the formation of glucose in a germinating seed due to some chemical change which takes place as the seed germinates, or to the imbibition of water? Why?

*g. Action of the diastase of malt upon starch.*

Make a thin starch paste by adding to boiling water a few drops of a mixture of dry starch and water. Cool the paste, and to it add a pinch of Merck's diastase of malt, which may be obtained of druggists. Let the test-tube stand in a warm place for a few minutes, then test its contents for the presence of glucose. Do you find any? Can diastase change starch to grape-sugar? If a diastasic ferment were present in a plant-cell, do you think that the starch in the cell might be changed to glucose?

*h. Osmosis.*

Take two dialyzers. Into the inner jar of one put some starch mixed with water; into the inner jar of the other put some grape-sugar dissolved in water. Into the outer jar of each put distilled water. After a few hours test the water in the outer jar of the first dialyzer for starch; that in the outer jar of the second for grape-sugar. Which dialyzes?

Review the experiments on the contents of

dry and of germinating seeds. Can you trace any connection between this experiment and the transfer of food-material from the storage leaves or cotyledons into the growing parts of the seedling plant?

Make sections of a cotyledon of a bean plant which has developed two or three pairs of leaves. Do you find as much starch present as before germination? Explain.

### B.—STEMS

*Material.*—Provide as specimens of the *woody* plants stems about two feet long of the horse-chestnut, elm, maple, willow, cherry, and pine. These should be taken during the winter or before the leaves expand in the spring. If to be examined during the early summer they may be preserved by being placed for a day in fifty per cent., and then kept permanently in eighty per cent. alcohol. To compare with these have fresh or alcoholic material bearing the expanded leaves and the flowers. It is possible to use dried specimens, but only as a last resort. For *herbaceous* stems use the *Begonia* or *Geranium*, *Tradescantia* (Wandering Jew), and strawberry, all of which may be had in the garden at certain seasons or at the greenhouse at any time of year. Have also a metric scale, dividers or calipers, forceps, hand-lens, razor, compound microscope, Schulze's solution, iodine, acetic acid carmine, Schulze's macerating mixture, acetic acid, hydrochloric acid, phloroglucin, sponge, bell-jar, small camel's-hair brush, India ink, and scalpel.

*Method of Examination.*—First study the arrange-

ment and shape of the branches on the plant. Remove some to the laboratory, and study their structure as detailed below. When not in use keep the material, if fresh, under large bell-jars or in closed boxes, with plenty of moisture and protected from evaporation. Examine alcoholic material in a mixture of equal parts of glycerine and fifty per cent. alcohol.

#### MORPHOLOGY

##### *Naked-eye Characters.*

Examine the stem of the horse-chestnut, and note the following characters:

- a. Shape.*—What is it? What variations do you find in different specimens? Is there a general shape for all of the branches on the tree? How does the shape of the branch compare with that of the main stem or trunk of the tree? How do you account for the bends in the stem? Draw.
- b. Size.*—What is the diameter of the specimen under examination? Is it the same throughout its whole length? How do you account for the facts observed?
- c. Color.*—What is the general color of the branch? To what is it due? Do you find any variations in color in different parts of the stem? How do you account for such?
- d. Structure.*—Notice that the stem consists of a series of sections or **internodes** connecting at joints or **nodes**.
  1. The **internode**.—What is the shape of an internode? The average length? Does the length vary in different parts of the stem? How do



you account for the results obtained? How much does the stem grow in length in one year? Is the growth even? How many internodes are there on the specimen examined? Notice the **leaf-scars**. Where are they found? How do you distinguish them from other parts of the stem? What is their position with reference to one another? Are all at the same distance apart? What is their shape? Examine the small points, the ends of the fibro-vascular bundles of the leaf, found in each scar. How many are there? Is the number constant? How are they arranged? What relation do the leaf-scars bear to the buds?

Examine the bark on an internode. Note its color and thickness. Notice the breaks in it on the lower internodes. Do you find them also on the upper ones? Why? In what direction do these breaks run? How do you account for them? Examine the small, wart-like points, the **lenticels**, on the bark. Do they have any regular arrangement? How many are there on an internode? Carefully peel off the bark to see whether the lenticels originate in the bark itself or in the tissues below. With a sharp knife or scalpel cut crosswise through the middle of the first internode of the stem; make also a longitudinal section through the middle of the internode, and note that the stem consists of the following layers, named in order from the outside towards the centre.

2. The **brown bark**.—In addition to the features already observed, note its thickness as compared with the other layers. Do you find any

decided variation in thickness? Does the bark completely cover the stem? From its characters would you judge it to be a *living* tissue? Hold a piece of bark up to the light to see if the bark is transparent. Examine bark from different parts of the stem with regard to this point. What do you consider to be the function of this layer?

3. The **green bark**.—How does it compare in thickness and texture with the outer bark? To what is its color probably due? Of what use may this layer be in this part of the plant?
4. The **bast**.—What is its color? How does it compare in thickness with the other layers? In what direction do its fibres run? Do you find indications of more than one layer? Of what use is the bast?
5. The **wood**.—How does it compare in color, texture, and thickness with the other layers? Examine under a lens to see the **annual rings** and the **medullary rays**. How many rings do you find? Are all of the same width? Why? How much does the stem increase in diameter in one year? Make cross-sections of other internodes of the same stem, and note the number of rings found in each. Is it the same for all? How do you account for the results? In what direction do the medullary rays run? With what parts do they connect? Of what use to the stem is the wood? Note how easily the bast separates from the wood.
6. The **pith**.—How much of the cross-section does it occupy? What is its texture? Does the pith

extend through the entire stem? In what part of the stem is the pith relatively greatest in amount? Of what use is it?

Make drawings of cross and longitudinal sections of the internode, showing the disposition of the tissues.

7. The **node**.—How is it formed? How do you distinguish it from the internode? How does it compare in length with the internode? Is the arrangement of tissues in the node the same as in the internode? What relation does the node bear to the leaf-scars? To the buds? To the side branches?

Examine and compare with the horse-chestnut stem stems taken from the elm, maple, willow, cherry, and pine, and make drawings showing the structures observed. Study the stems of herbaceous plants like the *Begonia*, *Tradescantia*, strawberry, etc. Make drawings of these stems.

#### *Microscopic Structure.*

Make cross-sections of the last internode of a branch of the horse-chestnut taken in the winter, mount some of the best in water or dilute glycerine, and study under the low power. Note the general arrangement of the tissues seen in the cross-section in the study of the gross anatomy of the stem—the **brown bark** or **cork**, enclosing a chlorophyll-bearing layer, the **cortical parenchyma**, within which comes a ring of thick-walled fibres of sclerenchyma, the **bast fibres**, this being intimately associated with the **soft bast** which shades into a ring of small cells, the **cambium**, with granular contents. Then comes the wood or **xylem**, some of whose ele-

ments show large openings. Note the appearance of the annual rings. In the centre lies the **pith** or **medulla**. Make a diagram showing the arrangement of all these parts. Put on the high power and study the structure of each of these tissues. Do you find an **epidermis**? If so, does it form a continuous layer? What is the structure of its cells? Contents? How many cells in thickness is the **cork-layer**? What is their shape? Are there any intercellular spaces? Do these cells have a definite arrangement? Do the walls of these cells vary in thickness? Compare the outer with the inner cork-cells. What do the cork-cells contain? Do you find any places where a mass of cork-cells has pushed through the epidermis and formed a lenticel? What is the shape of the cells of the cortical parenchyma? Compare their arrangement with that of the cork-cells. What contents have these parenchymatous cells? Do you find any **crystals**? If so, test them with acetic and hydrochloric acids. Of what are the crystals composed? How are the bast-fibres arranged? Is the ring of bast-fibres broken or continuous? What is their shape? Contents? How do you distinguish the **soft** from the **hard bast** or bast fibres? Note the arrangement of the cells of the cambium. Does it form a complete ring? What is the shape of its cells? How do their walls compare in thickness with those of the other cells in the section? Do you find that the cells of the tissues (xylem and soft bast) on each side of the cambium have the same arrangement as the cells of the latter? If so, explain. Note the arrangement of the ele-

ments of the xylem. Do you find that those with large cavities, or **vessels**, occur in definite places? Do you find any elements which run in radial lines, the **medullary rays**, from the pith to the cambium? Can you trace the rays beyond the cambium? What is the shape of the cells forming the medullary rays? Why are these rows of cells called "medullary rays"?

Draw a portion of the section showing all of the elements with their contents.

Study the shape, arrangement, and contents of the cells of the pith. Stain sections in Schulze's solution and in phloroglucin. Note particularly the color assumed by the elements of each tissue. Cut other sections two or three internodes below, and compare with those just examined. What gives the xylem the appearance of being composed of concentric rings, the annual rings? Do these rings vary in thickness as compared with one another? If so, to what is the difference due? Do different parts of the same ring vary in thickness? Why? Make drawings showing the cause of the ringed appearance. Cut other cross-sections through the upper and the lower end of the green stem of the current year, and compare with the others. How many fibro-vascular bundles can you find in the young stem? What changes take place in their number and position in the older stems? Using the proper reagents, especially phloroglucin, try to trace the change of the **cellulose** into the **lignified** elements of the xylem as the stem gets older. Make longitudinal sections and compare with the transverse sections. Draw.

Isolate the elements of the stem by using Schulze's macerating mixture. Draw several of each kind of element found.

Examine transverse and longitudinal sections through the growing point or *punctum vegetationis*. Section the other stems, and compare with that of the horse-chestnut and with one another. Notice particularly the **tracheides**, which form the greater part of the xylem in the pine stem. Draw several. Study especially the stems of the *Begonia* and *Tradescantia*.

Make drawings of all of the sections studied. What structural resemblances can you trace between any of these stems and the rhizome of the fern?

#### PHYSIOLOGY

##### a. *Movements.*

1. *Geotropism*.—Sow some seeds, as mustard, flax, peas, and beans, in the meshes of a coarse sponge, and keep the latter well moistened under a bell-jar, placed near a window, until the seeds germinate and the young stems appear. Notice particularly the direction of several of the stems of each kind of plant. With a fine camel's-hair brush, dipped into India ink, paint four lines at equal distances apart, dividing each stem into quarters, and running from the cotyledons down to the surface of the sponge. Turn the sponge upside down, being careful to keep the plants in the same position as regards light. Examine the plants both morning and afternoon for several days. Does the direction of the stems change? In what way? How soon can you detect, by the bending of the lines

on the stem, that the latter is moving? How long does it continue? In what part of the stem does the bending begin? Reverse the plants again. Do they again change their position?

2. *Heliotropism*.—Prepare another set of plants in the same manner. When they have become well started, revolve the sponge so as to place on the side towards the centre of the room those plants which were on the side towards the window, and *vice versa*. Does any change take place in the plants? If so, in what direction do they move? Do you find this to be a common phenomenon among plants?
3. *Twining*.—Raise some morning-glory plants in a flower-pot covered by a bell-jar, and when the plants are large enough stick into the soil by the side of each a slender stake about a foot long. Does the plant make use of the stake as a support? How high is the plant before it begins to twine? Do the plants *begin* to twine around the stake without first being put in contact with it? What is the first internode which begins the process? Do all of the plants turn in the same direction around the support?

*b. Direct observation of the ascent of water in the stem.*

Select a stem of *Tradescantia* having several long, straight internodes. Hold the stem under water, as in a wash-bowl, and with a razor divide it transversely near the lower end of the straight portion, being careful to keep the cut portion of the apical end under water. Then, still holding the stem under water, cut a piece

off each side of the lowest internode, leaving the latter in the form of a long, thin wedge, whose tip is so thin as to be transparent. With a rubber band loosely fasten the stem to the surface of a slide, remove the preparation from the water, being careful to keep the section well moistened, wipe the superfluous water off the slide, put on the cover-glass, and examine under a medium power. Find the ends of the spiral vessels in the fibro-vascular bundles, and look for small particles being carried into the mouths of these vessels. If no particles are seen, add a drop of water containing a little powdered indigo or carmine. Do the particles enter any other elements than the spiral vessels? How rapid is the flow of water into the vessels? With a quick stroke of a sharp razor cut off the leaves one by one, noting the rate of flow after the removal of each leaf. Does their removal have any influence on the rate of flow?

#### C.—Roots

The examination of the gross and microscopic structure of these organs and the devising of physiological experiments are left to the ingenuity of the student. The roots of the flowering plants differ so little from those of the fern and from the rhizome of the fern and the stems just examined, that the student ought readily to comprehend the resemblances and differences. As specimens to work upon it is suggested that several of the following be used: seedling maple, beet, Indian corn, onion, bean, ivy, mustard, oat, and pumpkin. Both fresh and alcoholic material should be used.



Let the student make the examination with a view to answering the following questions: What is the general structure of roots? What are the most noticeable structural differences between roots and stems? How do the vascular bundles of the former compare with those of the latter in arrangement and structure? How and where does the transition of root to stem take place? In what way and from what tissues do the root-hairs originate? How does the root grow in length? In thickness? Are the root-hairs actually attached to the particles of the soil in which the plant grows? In connection with this question the following experiment is suggested: In the bottom of a box about a foot square and three inches deep lay a piece of well-polished marble. Fill the box with clean, damp sand, and in the latter plant beans, peas, wheat, and corn. After two or three weeks, by which time the roots will have become well grown, empty the box, carefully wash the marble, and look for the "corrosion figures" made upon its surface by the roots of the seedlings. Can you give any explanation of this result?

#### D.—BUDS

*Material.*—The best material is to be had in spring before the buds expand, as then they are largest and most easily examined. A series of specimens should be made to illustrate the gradual growth of the buds of various plants. The collection of these specimens should be begun in the early summer, when the young buds of next season will be found forming in the axils of the leaves of the current year. Beginning with May, collect each month from the plants named hereafter a set of stems bearing thrifty buds. Put the stems for a day

into a saturated solution of picric acid in water, wash the stems for half an hour in thirty per cent. alcohol, then place them in fifty per cent. and seventy per cent. alcohol, each for a day, and keep permanently in eighty-five or ninety per cent. alcohol.

Provide buds of the horse-chestnut, lilac, hickory, tulip-tree, maple, elm, cherry, potato (tuberous portion), and onion.

Use will be made of the scalpel, hand-lens, compound microscope, dilute potash, dilute glycerine, Schulze's solution, acetic acid carmine, razor, watch-glasses, and metric scale.

*Method of Examination.*—Study first fresh, fully developed buds, then examine the set of preserved specimens. If these have been kept in strong alcohol, soak them for two or three hours in a mixture of three parts of fifty per cent. alcohol and one part of glycerine, and keep them moistened with this mixture while the examination is in progress. Section the buds in various directions for the study of both gross and minute anatomy.

#### MORPHOLOGY

##### *Naked-eye Characters.*

Examine the buds of the horse-chestnut in spring before they begin to swell, and again when they are partially expanded.

- a. *Position.*—At what places on the stem are buds formed? Do they occupy a constant position? Do you always find buds in this place? What determines their position? How many buds do you find on an internode? Do they occur singly or in pairs?

- b. Shape.*—What is the shape of the bud before it begins to expand? How does the shape change as expansion goes on? Of what use may this particular shape be?
- c. Size.*—Measure the length and diameter of the bud before it swells. As the bud expands, which increases the more, the length or the diameter? Do you find any especially small buds? If so, does their position correspond to that of the larger buds? How do you account for the presence of the small buds?

Draw several of the buds.

- d. Color.*—What is the color? Is the bud evenly colored? To what parts of the bud is the color confined? Of what use is this particular color?
- e. Structure.*
1. The **bud-scales**.—In what part of the bud are they found? Does this position have any relation to that of the leaf-scars on the stem? How many scales are there? What is their color? Are they evenly colored? What variations in size, shape, and color? What is the texture of the base of the scale? Of the top? Of the edge? How does the inner differ from the outer surface? Notice the **varnish** on the outside of the bud. Do you find it anywhere else than on the scales? Do you find it on all of the scales? On all parts of the scales? Of what use is it? Is the scale at all leaf-like in structure? In color? In shape? At what time of year are the scales formed? Do they

remain after the bud has expanded? What functions do the bud-scales perform?

Draw several of the bud-scales.

2. The **young leaves**.—In what position do you find them? How many are there? Is there any correspondence between the position of the leaves in the bud and that of the leaf-scars? Spread out one of the leaves. Does it resemble in any way the fully developed leaf? In what manner is the leaf folded in the bud, or, in other words, what form of **vernation** does it illustrate? Compare with the fern frond. Note the **down** covering the leaves. Of what use is it? At what time of year are the leaves formed? As the bud expands notice the manner and order in which the leaves appear. If the buds be examined before the leaves unfold, their expansion may be hastened by keeping the stems bearing the buds in a warm place and with their cut ends in water.

3. The **axis**.—Cut a bud in two lengthwise, and notice that the axis of the bud is the continuation of the stem. What structures do you find borne upon the axis? To what extent are these structures developed?

Make a diagram showing the structures borne by the axis.

In what respects does a bud resemble a seed? A stem? In what ways does it differ from a seed?

Examine buds from different kinds of trees, and compare with the horse-chestnut bud. Compare an onion with a bud.

Make drawings of the buds found on all of the stems examined.

*Microscopic Characters.*

Make longitudinal sections passing through the middle of the bud, and under the low power note the arrangement of the scales, young leaves, and flowers, if any. Under the high power study the more minute structure of the various parts, the course of the fibro-vascular bundles, the structure and development of the glandular hairs, the arrangement of cells at the growing point, etc.

Make transverse sections and compare with the others.

Draw sections of each bud examined.

**E.—LEAVES**

*Material.*—Fresh material is best, but pressed leaves or those preserved in alcohol may be used. To show the changes which take place during the season, particularly those which affect cell contents, provide leaves gathered in the spring, in midsummer, and in the autumn.

For the study of the gross anatomy use leafy branches from the morning-glory, violet or pansy, maple, dandelion, clover, horse-chestnut, elm, bean, locust, grass, pine, honeysuckle, oleander, strawberry, *Geranium*, *Nasturtium*, etc. In the physiological experiments entire plants of the *Geranium*, *Begonia*, *Fuchsia*, corn, primrose, and sensitive plant will be used.

The apparatus and instruments needed are scalpel, hand-lens, compound microscope, thread, metric scale, razor, Schulze's solution, phloroglucin, acetic acid carmine, strong alcohol, dilute iodine, vaseline, balance, bell-jar, half a yard of rubber cloth, small thistle tube, tin-foil, watch-glasses, four large tumblers, and two

pieces of cardboard, each sufficiently large to cover the tumblers.

*Method of Examination.*—Handle the fresh leaves as little as possible, and keep in a closed box all those which are not in actual use, for in some cases even a short exposure to the air causes the leaf to wither. Soak alcoholic material previous to examination for two or three hours in a mixture of equal parts of fifty per cent. alcohol and glycerine, and examine in the mixture.

#### MORPHOLOGY

##### *Gross Anatomy.*

Using the leafy branches of the plants named, study the following:

- a. *Arrangement or phyllotaxy.*—Are the leaves arranged alternately or opposite to each other on the stem? If the former, tie a fine thread around the base of the stalk of the lowest leaf. Revolve the stem between the fingers, and tie the thread in like manner around the stalk of the second leaf; again, around the third leaf, and so on, until the thread arrives at a leaf which stands directly above the first. Counting from the latter, what is the number of the leaf which stands over the first? How many revolutions of the stem has the thread made? Make a list of plants in which the leaves have this **spiral** or **alternate** arrangement. Make another list of plants whose leaves are **opposite**. Do you find any specimens in which three, four, or five leaves form a circle, called a **whorl** or **verticill**, around the stem? Make diagrams showing the leaf arrangement of each specimen examined.

*b. Structure.*—Notice that the leaf consists of a stalk, the **petiole**, which, in the case of **simple leaves**, bears a single flat expansion, the **blade**, or, as in **compound leaves**, several such expansions, the **leaflets**. Arrange all of your specimens in two groups, putting the simple leaves in one group and the compound in the other. Write a list of the specimens included in each group. Sometimes the petiole bears on its lower end a pair of expansions, the **stipules**. Make a list of all your specimens which bear stipules, *i. e.*, are **stipulate**; another list of the **exstipulate** leaves. In some cases no petiole is to be found. Make a list of such **sessile** leaves.

Study all the leaves with regard to the following points:

1. The **petiole**.—What is its shape? Length? Diameter? Is it marked by a **furrow** or **channel**? If so, on which side of the petiole is it? What is the nature of the surface of the petiole? What is the shape of its base?
2. The **blade** or **lamina**.—Examine first the simple leaves. Is the blade in all cases distinctly separated from the petiole? What is its color? What is the general shape of the blade? Does it consist of a single, entire piece, or is it variously lobed? What is the shape of the apex? Of the base? Of the margin? What is the texture of the leaf? Does the lower side differ from the upper?

Study the arrangement of the veins (*venation*) in the blade. At what point do the principal veins begin? Do you in all cases find a single large vein or **midrib** running from the

base to the apex of the blade? Have you any specimens in which a number of large veins of about the same size run lengthwise of the blade, thus illustrating **longitudinal** or **parallel venation**? Make a list. In which leaves do the most of the large veins unite to form a network, thus showing **reticulated** or **netted venation**? Make a list. In a third list put all of the leaves in which the veins radiate from the base to the margin of the blade, examples of **radiate**, **digitate**, or **palmate venation**. Can you trace any relation between the **venation** and the **outline** of the blade? Are the veins composed of more or less firm tissue than the rest of the blade? Do the veins form a support for the other tissues? Hold a leaf between yourself and the light, and look to see if the veins near the margin are so arranged as to offer resistance to tearing the soft tissues of the blade. Do you find any large areas into which the veins do not penetrate?

3. The **stipules**.—What is their shape? What variations in shape? How are they attached to the petiole? In what respects do stipules resemble the leaf-blade?

Draw each of the simple leaves, showing its exact shape and venation.

Examine the compound leaves in like manner and draw each.

Can you arrange any of the specimens in a series to show how compound might be derived from simple leaves?



*Microscopic Characters.*

Make cross and longitudinal sections of the various petioles, and compare them with one another and with sections of the stem. At least three cross-sections of each petiole should be made—the first at the base, the second near the middle, and the third near the apex. Note the tissues present, their arrangement, and the distribution of the fibro-vascular bundles. Study surface sections showing the epidermis.

Examine both surfaces of each leaf with the low power, noting the areas into which the small veins divide the soft tissues and the distribution of stomata. Make sections and study the structure of each leaf, noticing especially the arrangement and structure of the epidermal cells, of the green tissue or **mesophyll**—some of whose cells form the **palisade parenchyma**, next the epidermis—the contents of the various cells, and the arrangement and structure of the fibro-vascular bundles.

Make the drawings necessary to show the facts observed.

How many and which leaves show decided structural differences between the upper and lower surfaces? What are these differences in each case? What reasons can you give for the arrangement and contents of the palisade cells? Compare the structure of leaves with that of the fern frond examined, and with the thallus of *Marchantia*.

## PHYSIOLOGY

*A.—Transpiration.*

- a. Provide two large tumblers, a piece of cardboard large enough to cover the mouth of each, and a large leaf of primrose or *Geranium* or other suitable plant. Fill the first tumbler nearly full of water. In the card punch a hole, into which the petiole of the leaf will fit snugly, but will not be compressed. Put the card over the first tumbler, insert the petiole until its lower end projects into the water, then set the second tumbler inverted over the leaf and resting on the card. As a control experiment arrange without the leaf two other tumblers, supported by a piece of cardboard in which no hole has been made. Let the experiment stand for a few hours, then examine the upper tumbler in each case. Has any moisture condensed on the inside? Whence does the moisture come?
- b. From a *Geranium*, *Begonia*, *Fuchsia*, sunflower, or maple, cut a large, well-developed leaf, seal up the cut end of the petiole with vaseline, lay the leaf in one pan of a delicate balance, weigh the leaf accurately, and make a note of the weight. Let the leaf remain on the balance. How long before the pans change position? At the end of half an hour balance the leaf again. How much weight has it lost? Weigh the leaf again at the expiration of twenty-four hours. What is the loss? Has the leaf shrunk in size? What percentage of its weight has been lost by the evaporation of water from its tissues? Compare the weight of

water lost by a leaf freely exposed to the air with that lost by another leaf kept under a bell-jar or in a closed box.

- c. Take a small plant like a primrose or *begonia*, growing in a flower-pot, closely wrap the entire flower-pot and the lower portion of the stem of the plant in rubber cloth such as dentists use, thus preventing any evaporation of water from the surface of the soil or through the flower-pot. Insert through the rubber into the soil a small thistle tube, so that water may be supplied to the roots as needed. Weigh the plant as thus arranged and make a note of the weight. At the end of a day weigh again and compare with the previous weight. How much water is transpired by the leaves in twenty-four hours in an ordinary living-room? Does a collection of plants in a room help to keep the air moist? If the weather be favorable, set the plant out of doors overnight and find how much moisture is transpired. Compare this with the amount lost during the same number of hours of exposure to the sun. Judging from your experiments, what relations exist between the transpiration of water by plants and the humidity of the atmosphere?

*B.—Assimilation.*

With a piece of tin-foil wrap both surfaces of the lower end of a leaf of a *Geranium*, *Begonia*, or corn plant, so as to exclude the light from that part of the leaf. Let the plant stand for several days exposed to the sunlight. At the end of that time cut off the leaf, place it for a few

minutes in boiling water, then for a day, or until bleached, in strong alcohol. Then place the leaf in a weak alcoholic solution of iodine. Does the covered assume a color different from that of the uncovered portion? Explain the conditions of the experiment and the result.

*C.—Movements.*

If a *Clematis* be accessible, study the manner in which the vine climbs.

Note the position assumed at night by the leaflets of the clover, bean, and locust.

At the greenhouse procure vigorous specimens of the sensitive plant, and by various methods, which are left to the student to devise, test the irritability of the leaves and study their change of position.

If specimens of the sundew and of Venus's fly-trap can be had, study the movements of the trichomes and of the leaf-blade. If time permits, repeat some of the experiments detailed in Darwin's "Insectivorous Plants."

**F.—FLOWERS**

*Material.*—In the case of large plants, as the cherry and apple, bring to the laboratory some of the branches with the flowers attached rather than the individual flowers.

As other specimens, bring violets or pansies, buttercups, morning-glories, dandelions, peas, or beans.

For the study of the developing flower either fresh or alcoholic material may be used. Forceps, scalpel, hand-

lens, microscope, razor, watch-glasses, dilute iodine, and acetic acid carmine will be needed in the examination.

*Method of Examination.*—Study first the flower in its natural surroundings, especially the plants among which it grows, the time of year during which it is in blossom, and the kinds of insects found to visit it, for much of the significance of the structure, color, etc., of a flower is to be learned only from a study of its surroundings and of other parts of the plant.

#### MORPHOLOGY

##### *Naked-eye Characters.*

Take a cherry branch bearing several blossoms in various stages of expansion and study the following:

- a. *Arrangement or anthotaxy.*—On what part of the stem are the blossoms borne? What position do they have with regard to one another? Is this constant? How many do you find in a cluster? Is the number invariable? What is their position with regard to the leaves? Make a diagram indicating the position of the clusters on the stem; another, showing the position of the flowers in a cluster.
- b. *Shape.*—What is the shape of a mature but unopened bud? Draw. Of an expanded flower? Draw. What reasons can you give for these facts?
- c. *Size.*—What is the diameter of a mature bud? Of an expanded flower? Does the flower *grow* as it opens?
- d. *Color.*—What is the color of a perfect blossom?

Are there any variations from this color? Do all the parts of the flower have the same color? Is it always the same part that is colored? To what is the color due? Crush one of the colored parts by pinching it between the thumb and finger. Does the color change? Why? Does the color change as the flower gets older? Why? Of what use is the color?

*e. Odor.*—Has the flower a well-defined odor? Is it characteristic? Is it a strong odor? Agreeable or disagreeable? Is it confined to any particular part of the flower? Does it vary in intensity at different times of the day? Why? Of what use is it?

*f. Structure.*—Proceeding from below upwards make out the following parts:

1. The **pedicel** or flower-stalk.—What is its shape? Color? Length? Diameter? Do any of these vary in different flowers? Of what use are these various characters? Draw a pedicel detached from other parts.
2. The **flower proper**.—Note that it consists of certain organs arranged in groups. How are these organs distinguished from one another? What is the shape of the groups? In what manner are they attached to the pedicel? Note the expanded end, or **receptacle**, of the pedicel. Proceeding from without inward, make out the following parts of the flower proper:
  - (a) The **calyx** or green part.—On what part of the flower is it found? Is it evenly green in color? What is its shape? Of what is it made? Does it consist of distinct parts or **sepals**?

Note the **calyx-tube** and the **limb** or border. How do they differ? What is the diameter of the tube? Depth? The length of the lobes of the limb? Width? Is their size constant? How many lobes are there? Is the number constant? What position have these lobes in a bud? Why? Is it different in the expanded flower? Why? Note the character of the surface and margins of the parts of the calyx. Look for **nectar** in the calyx. What is its color? Consistence? Taste? Why is it in this part of the flower? Do you find any elsewhere? What is its use? Why is the base of the calyx cup-shaped? Of what use is the calyx? What becomes of the calyx as the flower becomes older and the fruit begins to form? Do you find in the ripe fruit any trace of the calyx? Are the parts of the calyx at all leaf-like in character? Draw a calyx as seen from above and as seen in longitudinal section.

- (b) The **corolla** or colored part.—What position does it occupy in the flower? What is its general shape? Of what use is the shape? Of how many parts or **petals** does the corolla consist? Does the number vary in different flowers? How does the number of petals correspond with the number of sepals? To what other parts are they attached? In what manner? What is their shape? Size? Color? Are all of the same shape? Of the same color? What is the use of the color? What is the character of the surface and margins of the petals? Of the apex, and base or **claw**? What is the structure of a petal? What position have the

petals with regard to the calyx lobes? In what respects do the petals resemble leaves? What position have the petals in unopened buds? What is the use of the corolla?

Draw the entire corolla and several of the petals.

- (c) The **androecium**, consisting of the **stamens**.—What is its position in the flower? Does it bear any resemblance to the calyx and corolla? Does every blossom contain an androecium? How many stamens in the androecium? How are they arranged with regard to the calyx and corolla and with regard to one another? Are they all of the same shape? Size? Color? Make a diagram of the androecium, showing the arrangement of its parts.

Examine a single stamen and make out the following parts:

- (1) The **filament** or stalk.—What is its shape? Length? Do you find variations in length? Can you give reasons for the same? What is its diameter? Color? Does it bear any outgrowths, such as hairs? If so, where are they? Why here?
- (2) The **anther**.—What is its position on the filament? Shape? Size? Color? To what is the color due? Make out the two parts, **pollen-sacs**, of the anther joined by the **connective**, also the **lines of dehiscence** along which the sacs split open. Can you trace any resemblances between the anther of a flower and the sporangium of a fern? Draw a stamen. Note the fine powder or **pollen** which comes out of the sacs. Put some of



the pollen on a slide without a cover-glass and examine with the low power.

- (d) The **gynæcium**, consisting of the **pistil**.—What relation does it bear to the other groups? Is it present in all of the blossoms? In what way does it resemble any of the other parts? Draw the gynæcium.

The pistil consists of the following parts named from below upwards:

- (1) The **ovary**, at the base of the pistil.—How much of the pistil does it form? What is its shape? Color? To what is it attached? Open it and notice the **seed** with its contained **ovule**. What is its structure? What part of the fruit does the wall of the ovary become? What part the seed? What changes in shape, size, color, and structure take place as ripening goes on?
- (2) The **style** or stalk of pistil.—What is its position? Shape? Size? Color? Compare with the filament of a stamen. Is the dividing line between style and ovary well marked? Why called "style"? What becomes of the style as the fruit ripens?
- (3) The **stigma** or top of style.—What is its shape? Size? Color? Of how many parts or **lobes** does it consist? Examine the surface of a mature stigma for pollen grains. What holds them in place? How do they get from the anther to the stigma? Why named "stigma"?

Compare with this flower others taken from the apple or pear tree, bean or pea, violet or pansy, buttercup and the dandelion, noticing especially

the various structural modifications which the parts undergo in each flower. Make a diagram of a cross-section of each flower, showing the number and position of all the parts, also a drawing twice natural size of one of each of the floral organs showing its shape and structure. Examine old flowers and young fruits of each plant, and trace the changes which take place in the parts of the flower. In which flowers do you find organs of the same kind grown together, illustrating **coalescence**? In which, organs of different kinds, **adnation**? In which do you find the parts of any group of organs unlike in shape, examples of **irregularity**? Endeavor to account for the various shapes, colors, arrangement of parts, etc., found. In what way does a flower resemble a bud? A leafy branch? Which of the flowers illustrate **symmetry**, *i. e.*, have the same number of parts in each group of organs, or have a certain number in some groups but a *multiple* of that number in the remaining groups?

#### *Microscopic Structure.*

Section the pedicel of the flower and compare its tissues, vascular bundles, etc., with those of the stem and of the petiole. Examine the sepals and petals and compare with one another and with leaves. Draw all of the sections. Study the structure of the filament and anther and examine the pollen. What is the shape of the pollen grains? Color? Do they bear any markings or projections on the surface? Do you find variations in any of these particulars? What is

the structure of a pollen grain? Does a pollen grain in any way resemble a fern spore? Draw several pollen grains. Examine sections through a young and through a mature anther. Does it resemble a sporangium? Is it at all leaf-like in structure? Study cross and longitudinal sections of the various parts of the pistil. Note particularly the structure of the ovary, the number and position of the ovules, the ridge or **placenta**, to which each of the latter is attached by the stalk or **funiculus**. In favorable longitudinal sections through an ovule try to find the central portion, the **nucellus**, surrounded by two coats, the inner or **primine** and the outer or **secundine**. Between the edges of the former look for an opening, the **micropyle**, and near the centre of the nucellus a large cell, the **embryo-sac**. In which direction does the micropyle point? Draw. Which of these parts are apparent in the mature seed? Is the pistil in any respect leaf-like?

Study very young flower buds, and endeavor to trace the origin and development of the various floral organs.

#### PHYSIOLOGY

##### *a. Fertilization.*

With a lens examine the pistils of various flowers, as lily, pumpkin, *Gloxinia*, etc., for pollen grains which have become attached to the stigma. Having found such a pistil, remove it and make longitudinal sections. Some of these will probably show growing down into the tissues of the style the tube emitted by the pollen grains. Trace the tubes as far as possible and make the

necessary sketches. Make longitudinal sections of the ovary to find pollen tubes penetrating the ovule.

Examine various flowers to answer the following questions: Is the pollen set free at the time the stigma becomes mature, which state is usually shown by a viscid secretion on its surface? Is the pollen in such a position and of such structure that it may fall or be blown upon the stigma? Do the stamens grow in such a position or are they of such shape that, if an insect were to alight upon the flower or to enter it, some of the pollen would be dusted upon or would adhere to his body? Is the pistil in such a position that a visiting insect would rub against it? If the flower secretes nectar, is it in such a place that, in order to get it, the insect must brush against stamens or pistil or both? Is the nectar freely exposed, or is it at the bottom of a tube, out of the reach of all insects except such as have a long proboscis, or is the entrance to the place where the nectar is stored guarded by hairs, bristles, etc.?

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## APPENDIX

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### A.—LIST OF REAGENTS, ETC.

1. *Acetic Acid.*

*Use.*—Clears opaque tissues and thick sections; swells cellulose walls and starch grains; dissolves crystals of calcium carbonate with effervescence. Dilute solutions bring out nuclei very clearly.

*Preparation.*—To prepare the dilute, or one per cent. aqueous solution, dissolve one gram of glacial acetic acid in ninety-nine cubic centimetres of distilled water.

2. *Acetic Acid Carmine (Schneider's).*

*Use.*—Stains fresh tissues rapidly; makes the nucleus show plainly.

*Preparation.*—From glacial acetic acid prepare a solution of forty-five per cent. strength. Heat this solution to the boiling-point, and while at this temperature add finely pulverized carmine until no more will dissolve. Filter the mixture and use it concentrated, or, better, diluted to one per cent. The latter strength stains more slowly than the former.

3. *Alcohol.*

*Use.*—Of all reagents alcohol is used most frequently, and in the largest amount. It is not only a hardening agent and a preservative for permanent preparations, but in the diluted form is used in the examination of preserved specimens; consequently, a large supply should be kept

on hand. To purchase alcohol at the regular market price, which is about two and a half dollars per gallon, entails upon the laboratory a serious expense, which, with little trouble, may be avoided. By act of Congress every incorporated educational institution is permitted to purchase alcohol free of the internal revenue tax when the alcohol is to be used for scientific purposes only. Before the alcohol may be withdrawn from the bonded warehouse in which it is stored, certain preliminaries must be performed. These are as follows: From the collector of internal revenue for your district learn where the nearest distillery is located; write to the distiller, specifying the amount of alcohol desired; in return you will receive a bond, an application for the withdrawal of alcohol from bond, and a gauger's receipt specifying the number, etc., of the package of alcohol which has been set aside for you; you will then fill out the application with the name of the institution, curator, etc., and forward it to the Secretary of the Treasury, along with the gauger's receipt and the bond, the latter having been signed previously before a notary-public, and in the presence of two witnesses, by the principal officer or curator of the institution and two sureties. In due time the distiller will receive from Washington a permit for the withdrawal from bond of the package of alcohol which you wish to purchase. This will then be forwarded you. By observing such procedure alcohol may be obtained for about seventy-five cents per gallon, or less than one-third the market price.

Absolute alcohol comes in one-pound bottles, and is used as a killing and hardening agent. Objects killed in this may be preserved in seventy per cent. alcohol.

*Preparation.*—As procured in the market or from the distillery, alcohol varies from ninety to ninety-five per cent. in strength. From this the various grades may be prepared approximately by mixing ninety-five per cent. alcohol and water in the proportions given in the following table:

Per cent.	Alcohol	Water	Per cent.	Alcohol	Water
84	6	1	48	1	1
82	5	1	45	1	1.25
78	4	1	42	1	1.5
75	3	1	35	1	2
67	2	1	30	1	3
62	1.5	1	22	1	4
60	1.25	1	18	1	5
55	1.1	1	—	—	—

#### 4. *Alcoholic Carminic Acid.*

*Use.*—Stains rapidly the protoplasm and nuclei of fresh or alcoholic specimens.

*Preparation.*—The crystals of carminic acid come in small vials. A sufficient number of the crystals may be added to alcohol of any strength until a solution of the desired depth of color has been produced.

#### 5. *Barium Hydrate.*

*Use.*—The solutions of barium hydrate are used to detect the presence of carbon dioxide, which unites with the barium hydrate and forms a white precipitate of barium carbonate.

*Preparation.*—Add barium oxide to distilled water until a saturated solution is formed.

#### 6. *Bristles.*

These are used to insert into and follow the course of blood-vessels, ducts, etc. Their ends should be guarded by a small knob of sealing-wax. It is convenient to have bristles of various lengths, colors, and degrees of stiffness. They may be obtained from manufacturers of paint-brushes.

#### 7. *Chloral Hydrate.*

*Use.*—Anæsthetic and antiseptic; clears pollen-grains, etc.

*Preparation.*—Add two grams of the crystals to ninety-eight cubic centimetres of distilled water. This solution may be added drop by drop to the water containing small animals which it is desired to anæsthetize.



8. *Chlor-iodide of Zinc (Schulze's Solution).*

*Use.*—A delicate test for cellulose; turns cellulose cell-walls and starch grains blue, protoplasm brown, and corky and lignified cell-walls brown.

*Preparation.*—To strong hydrochloric acid add some pure zinc until no more will dissolve. Set the solution in a warm place, add more zinc, and evaporate the fluid until it has a syrupy consistency. To the solution add crystals of potassium iodide until no more will dissolve, then saturate the mixture with metallic iodine. This gives a dark-brown, concentrated solution, which may be diluted until it has the color of sherry-wine by adding the requisite amount of a solution of one part of potassium iodide dissolved in twenty parts of water. Keep the solutions in a dark place.

9. *Chromic Acid.*

*Use.*—A one-half per cent. solution may be used for hardening tissues; strong aqueous solutions dissolve lignified and cellulose cell-walls.

*Preparation.*—Chromic acid comes in the form of red crystals. Make a four per cent. solution by dissolving four grams of the crystals in ninety-six cubic centimetres of distilled water. Dilute this solution as required. Keep the solutions of chromic acid in the dark.

10. *Copper Sulphate.*

*Use.*—Used with sodium hydrate as a test for glucose and for albuminous (proteid) substances.

*Preparation.*—Dissolve one gram of the crystals of copper sulphate in ninety-nine cubic centimetres of distilled water.

11. *Corrosive Sublimate.*

*Use.*—Is a hardening agent, and kills small animals almost instantaneously. Do not handle with steel instruments specimens killed in corrosive sublimate.

*Preparation.*—Saturate distilled water with mercuric chlo-

ride. One litre of water will dissolve about seventy grams of mercuric chloride.

12. *DeLafield's Hæmatoxylin.*

*Use.*—One of the best of stains for alcoholic material.

Must not be used until specimen has been freed from any acids that may have been used in the process of preservation. In staining add a few drops of the solution to distilled water until the desired depth of color is obtained, then immerse the specimen. Dilute solutions stain much more satisfactorily than concentrated, though longer time is required.

*Preparation.*—Dissolve four grams of hæmatoxylyn crystals in twenty-five cubic centimetres of ninety per cent. alcohol. Saturate four hundred cubic centimetres of distilled water with ammonia alum; to this add the first solution. Let the mixture stand exposed to the light and air in an open bottle for three or four days; then filter, and to it add one hundred cubic centimetres of glycerine and one hundred cubic centimetres of methyl alcohol. Let the mixture stand for a time, then filter again, and keep in a stoppered bottle. The stain works best if allowed to ripen for two or three months before using.

13. *Dissecting-Dishes.*

These may be of tin or earthenware. If the latter, ordinary vegetable dishes will serve. A convenient tin dish is a foot long, six inches wide on the bottom, seven or eight inches wide on top, and two and one-half to three inches deep. The bottom of the dishes should be covered by a layer one-quarter to one-half inch thick of beeswax or paraffin which has been mixed with lamp-black. A dark background is thus formed against which the delicate tissues of the various specimens show well, and the parts may be displayed by pinning them out on the wax. It will be found that the pins will hold better in beeswax than in paraffin. Before pouring the melted wax into the dishes, put into the bottom of each a num-

ber of large shot to weight the wax, otherwise, as it separates from the sides of the dish in cooling, it will float when water is poured into the dish. If tin dishes be used, smear some of the melted wax over the inside of each to prevent rusting. The dishes should be washed and thoroughly dried after being used.

14. *Dissecting-Needles.*

These are invaluable in the examination of small organisms, pieces of tissue, etc. They may easily be made by thrusting the eye end of sewing-needles into pen-holders made of soft wood. It is well to have several pairs made with needles of different sizes.

15. *Dissecting-Trays.*

Convenient trays may be made of deal boards measuring eighteen by twelve inches. They may have a narrow moulding or a deep groove at the edge to catch the fluids and prevent them from soiling the tables. The trays should be thoroughly oiled before being used, as the oil prevents the wood from absorbing fluids and facilitates cleaning. If desired, the trays may be stained black with a solution of logwood before being oiled. They should be thoroughly cleansed each time after using.

16. *Eosin.*

*Use.*—Stain for alcoholic or fresh tissues.

*Preparation.*—Prepare the alcoholic solution by dissolving one gram of eosin in ninety-nine cubic centimetres of ninety-five per cent. alcohol. When using, add this solution to alcohol in the proportion of one drop of the former to twenty drops of the latter. The aqueous solution is prepared by dissolving in distilled water sufficient eosin to produce the depth of color desired.

17. *Glycerine.*

*Use.*—Clears tissues; serves as a temporary mounting medium.

*Preparation.*—Either concentrated glycerine or the dilute solution may be used for mounting. The latter is made by mixing equal parts of glycerine and distilled water.

18. *Hydrochloric or Muriatic Acid.*

*Use.*—Dissolves crystals of calcium carbonate and of calcium oxalate, the former with the latter without effervescence; decalcifies; macerates; turns lignified cell-walls yellow.

*Preparation.*—The concentrated acid, which is perfectly colorless, may be diluted with distilled water to the various strengths needed. Keep in glass-stoppered bottles.

19. *Injection Masses.*

*Starch.*—The starch injection mass serves well for coarse anatomical work, and is easily managed. The mass itself consists of one volume of dry starch, one volume of a two and a half per cent. aqueous solution of chloral hydrate, one-fourth volume of ninety-five per cent. alcohol, and one-fourth volume of the color. The last is prepared by mixing together equal volumes of the dry color—*e. g.*, vermilion or soluble Prussian blue, glycerine, and ninety-five per cent. alcohol. These ingredients should be thoroughly ground together in a mortar, and strained through fine muslin to remove the lumps which might clog the cannulæ of the syringe. This color mixture may be kept permanently in a closed bottle, and added to the starch mass as desired.

*Gum-arabic.*—Make a thick paste by dissolving gum-arabic in water to which has been added sufficient soluble Prussian blue to produce the desired depth of color; strain the paste through fine muslin; place the injected specimen in alcohol to harden the paste.

20. *Iodine.*

*Use.*—Colors starch blue, proteid substances (protoplasm, etc.) brown, cellular cell-walls light-yellow, cuticularized and lignified cell-walls yellow.

*Preparation.*—Dissolve to saturation crystals of potassium

iodide in distilled water ; then saturate the solution with metallic iodine. Dilute this with one to three times its bulk of distilled water as desired. Keep all solutions of iodine in the dark.

21. *Magenta.*

*Use.*—Stains fresh tissues.

*Preparation.*—Dissolve one gram of crystallized magenta (roseine) in ninety-nine cubic centimetres of ninety-five per cent. alcohol. Dilute this to any strength needed by adding distilled water.

22. *Mayer's Pepsin Solution.*

*Use.*—Culture-medium for yeast, etc.

*Preparation.*—The following formula is taken from Huxley and Martin's "Practical Biology":

Fifteen per cent. solution of sugar candy	20	cubic centimetres
Dihydropotassic phosphate . . . . .	0.1	gram
Calcic phosphate . . . . .	0.1	"
Magnesian sulphate . . . . .	0.1	"
Pepsin . . . . .	0.28	"

23. *Millon's Reagent.*

*Use.*—Turns albuminous (proteid) substances red.

*Preparation.*—Dissolve metallic mercury in its own weight of strong nitric acid. This is best done by pouring the acid upon the mercury in a beaker. As suffocating fumes and considerable heat are generated, it is best to place the beaker on a cloth under a ventilating hood, or on a ledge outside the window. When the mercury is entirely dissolved add to the solution twice its volume of distilled water. Let the mixture stand for a few hours, then decant it into a glass-stoppered bottle.

24. *Moist Chamber.*

A very simple form of moist chamber in which to examine cultures of spores, pollen-grains, etc., is made in the following manner: From a piece of smooth paste-

board of medium thickness cut a square which does not exceed a glass slide in width; in the centre of the square cut a circle whose diameter is somewhat less than that of a cover-glass; immerse the frame thus made in strong alcohol for a few hours in order to kill any organisms that may be attached to the pasteboard, otherwise they may later become active and vitiate the culture; when ready to start the culture remove the frame from the alcohol and soak it in distilled water until thoroughly saturated, then lay the frame on the glass slide; in the centre of the cover-slip put a drop of the culture-fluid, which has previously been sterilized by boiling; sow in the drop a few of the spores which are to be cultivated; then, with the drop hanging from the under side, place the cover-glass on the pasteboard frame, and keep the preparation under a bell-jar with a dish of water to keep the atmosphere moist and to prevent the pasteboard from drying; examine the pasteboard frequently, and if it shows signs of drying moisten its edges with distilled water. It is convenient to keep in strong alcohol a permanent supply of these frames. Moist chambers may also be made by using glass rings, which are furnished by dealers in microscopical supplies.

25. *Müller's Fluid.*

*Use.*—Hardening reagent. Specimens may be left in it from two to several weeks.

*Preparation.*—In one litre of water dissolve twenty-five grams of bichromate of potash and ten grams of sodium sulphate.

26. *Nitric Acid.*

*Use.*—Hardens nerve, macerates muscle, and decalcifies osseous tissues; colors proteids and cuticularized cell-walls yellow; swells cellulose and lignified cell-walls.

*Preparation.*—Make dilute solutions from the colorless concentrated acid. Keep in glass-stoppered bottles.

27. *Osmic Acid.*

*Use.*—Kills living protoplasm—*e. g.*, Protozoa, etc., instantaneously; fixes nuclei; and stains fats and oil black.

*Preparation.*—Crystals of osmic acid come in small tubes, each containing one gram. One of these tubes may be broken in a bottle containing ninety-nine cubic centimetres of distilled water. This gives a one per cent. solution which may be diluted. The bottle should have a ground-glass stopper and be kept in a dark place, or wrapped with thick, opaque paper.

28. *Pasteur's Solutions.*

*Use.*—Culture-media for yeast, mould, spores of fungi, etc.

*Preparation.*—The following formula for making the solution “with sugar” is given in Huxley and Martin’s “Practical Biology”:

Potassium phosphate . . . . .	20 parts
Calcium phosphate . . . . .	2 “
✓Magnesium sulphate . . . . .	2 “
✓Ammonium tartrate . . . . .	100 “
[Cane-sugar] . . . . .	1,500 “
Water . . . . .	8,376 “
	<hr/>
	10,000 parts

The solution “without sugar” is the same as the above with the sugar omitted.

29. *Perenyi's Fluid.*

*Use.*—Kills small animals, eggs, etc., instantaneously. Objects may be left in it from two to five hours, then transferred to seventy per cent. alcohol for a day, then to ninety per cent. alcohol.

*Preparation.*—Mix together four parts of a ten per cent. solution of nitric acid, three parts of a one-half per cent. solution of chromic acid, and three parts of ninety per cent. alcohol.

30. *Picric Acid.*

*Use.*—Hardens tissues in two or three hours to a day, according to the size of the piece, and decalcifies bony structures. Remove the excess of picric acid by washing in alcohol, and stain picric acid preparations in alcoholic stains. Transfer specimens fixed in picric acid to seventy-five per cent., and then to ninety-five per cent. alcohol.

*Preparation.*—A saturated aqueous solution is made by adding crystals of the acid to distilled water until no more will dissolve.

31. *Picro-sulphuric Acid (Kleinenberg's).*

*Use.*—Hardens and decalcifies. To harden objects place them in the fluid for three to five hours, transfer to seventy per cent. alcohol for five to six hours, then place in ninety per cent. alcohol, and change the latter as often as it becomes discolored.

*Preparation.*—To one hundred volumes of a saturated solution of picric acid in distilled water add two volumes of concentrated sulphuric acid. Filter this mixture and dilute it with three times its volume of distilled water.

32. *Pith.*

Bunches of pith may be obtained from dealers in watch-makers' supplies.

33. *Potash.*

*Use.*—Clears vegetable tissues by causing the cell-walls, starch grains, etc., to swell, and the proteid crystalloids and aleurone to dissolve.

*Preparation.*—Potassium hydroxide comes in the form of sticks. These usually have an opaque white coating of potassium carbonate, which should be dissolved off, and only the central, transparent part of the stick used. Make a concentrated solution by dissolving twenty-five grams of potassium hydroxide in seventy-five cubic centimetres of distilled water. Make the five per cent. (dilute)



solution by dissolving five grams of potassium hydroxide in ninety-five cubic centimetres of distilled water. Keep potash solutions in bottles with ground-glass stoppers, and smear the latter with vaseline to prevent them from becoming fastened in the necks of the bottles.

#### 34. *Reagent Bottles.*

Each table should be supplied with at least one set of the common reagents and stains, put up in bottles holding two or more ounces, according to the number of students working at the table. If the bottles be circular they may be set into a tray made by boring holes of the required diameter in a piece of plank an inch or more in thickness. The tray will avoid the necessity of a set of shelves, which is often in the way, and will keep the bottles in a compact space. Each bottle may be closed by a bulb stopper which carries a glass pipette and serves at the same time for a dropper. These "Standard Medicine Droppers" come in boxes containing one dozen each, and may be obtained of J. J. Requa, New York. Each bottle should also have a label, giving the name, composition, and use of its contents.

#### 35. *Sachs's Food Solution for Green Plants.*

*Use.*—A culture-medium for green plants.

*Preparation.*

Distilled water . . . . .	1000 cubic centimetres
Potassium nitrate . . . . .	1 gram
Sodium chloride (common salt) . . . . .	0.5 "
Calcium sulphate (gypsum) . . . . .	0.5 "
Magnesium sulphate . . . . .	0.5 "
Calcium phosphate (pulverized) . . . . .	0.5 "

To the above mixture add five or six drops of a weak solution of chloride of iron.

#### 36. *Salt Solutions.*

*Use.*—The strong solutions are used as plasmolyzing agents; the weak, to form a neutral medium in which

living tissues may be examined without undergoing the changes caused by pure water.

*Preparation.*—The strong solution (twenty per cent.) may be made by dissolving twenty grams of common table salt in eighty cubic centimetres of distilled water, and from this weaker solutions may be prepared. The “normal” (three-quarter per cent.) salt solution is made by adding seven and a half grams of salt to one litre of distilled water.

37. *Schulze's Macerating Mixture.*

*Use.*—Separates the constituent elements of vegetable tissues by dissolving the middle lamella. Used cold the mixture gives better results, but works more slowly than when warmed. Do not expose the microscope to the fumes given off.

*Preparation.*—Dissolve one gram of potassium chlorate in fifty cubic centimetres of nitric acid.

38. *Sea-water (artificial).*

The following formula gives an artificial sea-water in which starfishes, lobsters, etc., may be kept alive for some time :

Water . . . . .	3 to 4 litres
Sodium chloride (salt) . . . . .	81 grams
Magnesium sulphate . . . . .	7 “
Magnesium chloride . . . . .	10 “
Potassium chloride . . . . .	2 “

This water should frequently be aerated by pouring from a height into the tank.

39. *Silver Nitrate.*

*Use.*—A stain for fibrous tissues. The tissue to be stained is spread upon the slide in a drop of the solution, and exposed to the sunlight until a brown color is assumed. The preparation is then washed in distilled water.

*Preparation.*—Dissolve one-half gram of the crystals of silver nitrate in one hundred cubic centimetres of distilled water. Keep in the dark in a glass-stoppered bottle.

40. *Sodium Hydrate.*

*Use.*—With copper sulphate it forms a test for glucose.

*Preparation.*—Dissolve the sticks of sodium hydrate in three or four times their weight of distilled water. Keep in a bottle with a glass stopper, the latter having been smeared with vaseline to prevent sticking.

41. *Sugar.*

*Use.*—Concentrated solutions plasmolyze living cells; used also as a culture medium for yeast; and to cause the germination of pollen-grains.

*Preparation.*—To make the twenty per cent. solution dissolve twenty grams of granulated sugar in eighty cubic centimetres of distilled water. From this the ten per cent. and two per cent. solutions may be made by dilution.

42. *Sulphuric Acid.*

*Use.*—The concentrated acid dissolves cellulose cell-walls and swells starch grains; turns blue those cellulose cell-walls which have previously been saturated with a solution of iodine. For the last reaction dilute solutions are preferable.

*Preparation.*—Dilute the concentrated colorless acid to the strength desired. Make the dilute by mixing equal parts of the concentrated solution and distilled water; the seventy-five per cent. solution by mixing seventy-five parts of the acid with twenty-five parts of distilled water. Keep in bottles with glass stoppers.

43. *Warm Stage.*

A simple warm stage consists of a T-shaped piece of sheet copper six inches long by three inches wide. In the middle of the cross-piece is punched a hole whose diameter is slightly greater than that of a cover-glass. When used the warm stage is placed on the stage of the microscope with the tongue projecting forward, and the hole over the opening in the stage. On the cross-piece is laid the slide, the whole being held securely in place

by the clips on the stage. On or by the side of the cover-glass is laid a small piece of paraffin, which melts at the temperature at which the object is to be studied. Let the tongue of the warm stage project into the flame of an alcohol lamp. The tongue of metal will conduct the heat back to the preparation on the slide, the temperature of the latter being indicated by the condition of the paraffin, which should be kept at the melting-point, but not allowed to become fluid. If the stage become too hot, cool it by removing the lamp for a time.

44. *Wickersheimer's Fluid.*

*Use.*—Small animals—*e. g.*, frogs—may be immersed in this fluid for a week or two, then taken out and dried in the air. Lungs, blood-vessels, and intestines should be filled with the fluid, though this is not necessary in the case of animals as small as the frog. The muscles, tendons, and ligaments remain soft and flexible, and the natural color of the organs is frequently well preserved.

*Preparation.*—In three thousand cubic centimetres of boiling water dissolve one hundred grams of alum, twenty-five grams of common salt, twelve grams of potassium nitrate, sixty grams of potash, and ten grams of arsenic trioxide. Cool and filter the mixture. Then to each ten litres of the above mixture add four litres of glycerine and one litre of ninety to ninety-five per cent. alcohol.

## B.—WORKS OF REFERENCE

Under its proper head, in the following list, will be found the titles of books or articles, some of which the student should consult in connection with the examination of each organism. The list is not intended to be complete, and includes only such works as are likely to be found in almost every library. For the sake of convenience each title is numbered, and is given in full only when first mentioned.

### MICROSCOPICAL MANIPULATION AND LABORATORY METHODS

1. Beale.—“How to Work with the Microscope,” London, 1880. Structure and methods.
2. Behrens.—“Guide to the Microscope in Botany,” Boston, 1885. Structure and methods.
3. Bower and Vines.—“A Course of Practical Instruction in Botany,” New York, 1889. Methods.
4. Carpenter.—“The Microscope and Its Revelations,” revised by Dallinger, Philadelphia, 1891. Structure and methods.
5. Fearnley.—“A Course in Elementary Practical Histology,” New York, 1887. Laboratory and microscopical methods.
6. Frey.—“The Microscope and Microscopical Technology,” New York, 1872. Structure and methods.
7. Gage.—“The Microscope and Histology,” Part I, Ithaca, 1891. Structure and methods.
8. Goodale. — “Physiological Botany,” New York, 1885. Methods.
9. Howes.—“Atlas of Practical Elementary Biology,” London, 1885. Methods.

10. Huxley and Martin.—“A Course of Elementary Instruction in Practical Biology,” revised by Howes and Scott, New York, 1889. Microscopical and laboratory methods.
11. Lee.—“Microtometist's Vade Mecum,” Philadelphia, 1890. Methods.
12. Marshall and Hurst.—“Practical Zoölogy,” London, 1888. Microscopical and laboratory methods.
13. Poulsen.—“Botanical Micro-Chemistry,” Boston, 1886. Methods.
14. Schäfer.—“The Essentials of Histology,” Third edition, Philadelphia, 1892. Methods.
15. Strasburger.—“Hand-book of Practical Botany,” translated by Hillhouse, New York, 1889. Methods.
16. Whitman.—“Methods in Microscopical Anatomy and Embryology,” Boston, 1885. Methods.
17. Wilder and Gage.—“Anatomical Technology,” New York, 1886. Laboratory methods.

## GENERAL WORKS ON ZOÖLOGY

18. Claus and Sedgwick.—“Text-book of Zoölogy,” two volumes, New York, 1886.
19. Gegenbaur.—“Elements of Comparative Anatomy,” London, 1878.
20. Huxley.—“A Manual of the Anatomy of Vertebrated Animals,” New York, 1883.
21. Huxley.—“A Manual of the Anatomy of Invertebrated Animals,” New York, 1883.
22. Lang.—“Text-Book of Comparative Anatomy,” Part I, New York, 1891.
- 22a. Morgan.—“Animal Biology,” London, 1889.
23. Orton.—“Comparative Zoölogy,” New York, 1882.
24. Packard.—“Zoölogy for High-Schools and Colleges,” New York, 1889.
25. Rolleston and Jackson.—“Forms of Animal Life,” Oxford, 1888.
26. “Standard Natural History,” six volumes, Boston, 1885.
27. Thompson.—“Outlines of Zoölogy,” New York, 1892.

## GENERAL WORKS ON BOTANY

28. Bessey.—“Botany for High-Schools and Colleges,” New York, 1889.
29. Bower and Vines.—See No. 3 this list.
30. Campbell.—“Elements of Structural and Systematic Botany,” Boston, 1890.
31. Goebel.—“Outlines of Classification and of Special Morphology of Plants,” Oxford, 1887.
32. Goodale.—See No. 8 this list.
33. Gray.—“Structural Botany,” New York, 1880.
34. Sachs.—“Text-Book of Botany,” Oxford, 1886.
35. Sachs.—“Lectures on the Physiology of Plants,” Oxford, 1887.
36. Strasburger.—See No. 15 this list.
37. Vines.—“Lectures on the Physiology of Plants,” Cambridge, 1886.

## PROTOZOA

38. Claus and Sedgwick.—See No. 18 this list, vol. i.
39. Huxley.—See No. 21 this list.
40. Kent.—“Manual of the Infusoria,” London, 1880.
41. Lang.—See No. 22 this list.
42. Lankester.—Article “Protozoa,” in “Encyclopædia Britannica,” Ninth edition. Reprinted in “Zoölogical Articles,” New York, 1892.
- 42a. Morgan.—See No. 22a this list.
43. Packard.—See No. 24 this list.
44. Parker.—“Lessons in Elementary Biology,” New York, 1893.
45. Rolleston and Jackson.—See No. 25 this list.
46. “Standard Natural History.”—See No. 26 this list, vol. i.
47. Thompson.—See No. 27 this list.

## AMCERA

48. Brooks.—“Hand-book of Invertebrate Zoölogy,” Boston, 1882.

49. Bumpus.—“A Laboratory Course of Invertebrate Zoölogy,” Providence, 1892.
50. Claus and Sedgwick.—See No. 18 this list, vol. i.
51. Davis.—“A Text-book of Biology,” Philadelphia, 1888.
52. Foster.—“A Text-book of Physiology,” five volumes, Sixth edition, New York, 1893. Introduction.
53. Griffiths.—“Physiology of the Invertebrata,” London, 1892.
54. Howes.—See No. 9 this list.
55. Huxley.—See No. 21 this list.
56. Huxley.—Lecture on a “Piece of Chalk” in “Lay Sermons, Addresses, and Reviews,” New York, 1871. Also Lecture on “The Physical Basis of Life,” in same.
57. Huxley and Martin.—See No. 10 this list.
58. Lang.—See No. 22 this list.
59. Lankester.—See No. 42 this list.
60. Leidy.—“Fresh-water Rhizopods of North America,” in “U. S. Geological Survey of the Territories,” vol. xii., Washington, 1879.
61. Marshall and Hurst.—See No. 12 this list.
- 61a. Morgan.—See No. 22a this list.
62. Packard.—See No. 24 this list.
63. Parker.—See No. 44 this list.
64. Rolleston and Jackson.—See No. 25 this list.
65. “Standard Natural History.”—See No. 26 this list, vol. i.
66. Thompson.—See No. 27 this list.

## PARAMECIUM

67. Brooks.—See No. 48 this list.
68. Bumpus.—See No. 49 this list.
69. Claus and Sedgwick.—See No. 18 this list.
70. Huxley.—See No. 21 this list.
71. Kent.—See No. 40 this list.
72. Lang.—See No. 22 this list.
73. Lankester.—See No. 42 this list.
74. Marshall and Hurst.—See No. 12 this list.
- 74a. Morgan.—See No. 22a this list.
75. Packard.—See No. 24 this list.



- 76. Parker.—See No. 44 this list.
- 77. Rolleston and Jackson.—See No. 25 this list.
- 78. "Standard Natural History."—See No. 26 this list, vol. i.
- 79. Thompson.—See No. 27 this list.

## VORTICELLA

(Same as for "Paramecium") also

- 80. Davis.—See No. 51 this list.
- 81. Howes.—See No. 9 this list.
- 82. Huxley and Martin.—See No. 10 this list.

## SALIVARY CORPUSCLES

- 83. Foster.—See No. 52 this list.
- 84. Landois and Stirling.—"Text-book of Human Physiology," Philadelphia, 1889.
- 85. Schäfer.—See No. 14 this list.

## BLOOD CORPUSCLES

- 86. Davis.—See No. 51 this list.
- 87. Foster.—See No. 52 this list.
- 88. Howes.—See No. 9 this list.
- 89. Huxley and Martin.—See No. 10 this list.
- 90. Landois and Stirling.—See No. 84 this list.
- 91. Marshall.—"The Frog," Fourth edition, London, 1891.
- 91a. Morgan.—See No. 22a this list.
- 92. Schäfer.—See No. 14 this list.

## CILIATED CELLS

- 93. Davis.—See No. 51 this list.
- 94. Foster.—See No. 52 this list.
- 95. Howes.—See No. 9 this list.
- 96. Huxley and Martin.—See No. 10 this list.
- 97. Landois and Stirling.—See No. 84 this list.
- 98. Lang.—See No. 22 this list.
- 99. Marshall.—See No. 91 this list.
- 100. Schäfer.—See No. 14 this list.

## YEAST

101. Davis.—See No. 51 this list.
102. De Bary.—“Lectures on Bacteria,” Oxford, 1887.
103. De Bary.—“Comparative Morphology and Biology of Fungi, Mycetozoa, and Bacteria,” Oxford, 1887.
104. Howes.—See No. 9 this list.
105. Huxley.—Lecture on “Yeast” in “Critiques and Addresses,” New York, 1873.
106. Huxley and Martin.—See No. 10 this list.
107. Parker.—See No. 44 this list.
108. Pasteur.—“Studies on Fermentation,” London, 1879.
109. Schützenberger.—“Fermentation,” in “International Scientific Series,” New York, 1876.
110. Trouessart.—“Microbes, Ferments, and Moulds,” in “International Scientific Series,” New York, 1886.
111. Woodhead.—“Bacteria and their Products,” New York, 1891.

## GREEN SLIME

112. Arthur, Barnes, and Coulter.—“Hand-book of Plant Dissection,” New York, 1886.
113. Davis.—See No. 51 this list.
114. Goebel.—See No. 31 this list.
115. Howes.—See No. 9 this list.
116. Huxley and Martin.—See No. 10 this list.
117. Parker.—See No. 44 this list.
118. Sachs.—See No. 34 this list.

## SPORES OF FUNGI

119. Cooke and Berkeley.—“Fungi,” in “International Scientific Series,” New York, 1880.
120. De Bary.—See No. 103 this list.
121. Goebel.—See No. 31 this list.

## POLLEN-GRAINS

122. Bower and Vines.—See No. 3 this list.
123. Campbell.—See No. 30 this list.

- 124. Sachs.—See No. 35 this list.
- 125. Strasburger.—See No. 15 this list.

## SPIROGYRA

- 126. Arthur, Barnes, and Coulter.—See No. 112 this list.
- 127. Bower and Vines.—See No. 3 this list.
- 128. Campbell.—See No. 30 this list.
- 129. Cooke.—“Introduction to Fresh-water Algæ,” in “International Scientific Series,” London, 1890.
- 130. Davis.—See No. 51 this list.
- 131. Goebel.—See No. 31 this list.
- 132. Howes.—See No. 9 this list.
- 133. Huxley and Martin.—See No. 10 this list.
- 134. Parker.—See No. 44 this list.
- 135. Sachs.—See No. 34 this list.
- 136. Strasburger.—See No. 15 this list.
- 137. Wolle.—“Fresh-water Algæ of the United States,” Bethlehem, Pa., 1887.

## SPONGES

- 138. Brooks.—See No. 48 this list.
- 139. Bumpus.—See No. 49 this list.
- 140. Claus and Sedgwick.—See No. 18 this list, vol. i.
- 141. Griffiths.—See No. 53 this list.
- 142. Huxley.—See No. 21 this list.
- 143. Hyatt.—“Commercial and other Sponges,” in “Guides for Science Teaching,” No. III., Boston, 1883.
- 144. Lang.—See No. 22 this list.
- 145. Sollas.—Article “Sponges,” in “Encyclopædia Britannica,” Ninth edition. Republished in “Zoölogical Articles.” See No. 42 this list.
- 146. “Standard Natural History.”—See No. 26 this list, vol. i.

## HYDRA

- 147. Claus and Sedgwick.—See No. 18 this list, vol. i.
- 148. Davis.—See No. 51 this list.
- 149. Gegenbaur.—See No. 19 this list.

- 150. Griffiths.—See No. 53 this list.
- 151. Howes.—See No. 9 this list.
- 152. Huxley.—“On Corals and Coral Reefs,” in “Critiques and Addresses,” New York, 1873.
- 153. Huxley.—See No. 21 this list.
- 154. Huxley and Martin.—See No. 10 this list.
- 155. Hyatt.—“Common Hydroids, Corals, and Echinoderms,” in “Guides for Science Teaching,” No. V., Boston, 1875.
- 156. Lankester.—Article “Hydrozoa,” in “Encyclopædia Britannica,” Ninth edition. Republished in “Zoölogical Articles.” See No. 42 this list.
- 157. Marshall and Hurst.—See No. 12 this list.
- 157a. Morgan.—See No. 22a this list.
- 158. Parker.—See No. 44 this list.
- 159. “Standard Natural History.”—See No. 26 this list, vol. i.

## CAMPANULARIAN HYDROID

- 160. Agassiz, Mrs.—“A First Lesson in Natural History,” in “Guides for Science Teaching,” No. IV., Boston, 1886.
- 161. Brooks.—See No. 48 this list.
- 162. Bumpus.—See No. 49 this list.
- 163. Claus and Sedgwick.—See No. 18 this list, vol. i.
- 164. Gegenbaur.—See No. 19 this list.
- 165. Griffiths.—See No. 53 this list.
- 166. Huxley.—See No. 21 this list.
- 167. Hyatt.—See No. 155 this list.
- 168. Lang.—See No. 22 this list.
- 169. Lankester.—See No. 156 this list.
- 170. Parker.—See No. 44 this list.
- 171. “Standard Natural History.”—See No. 26 this list, vol. i.

## STARFISH

- 172. Agassiz, Mrs.—See No. 160 this list.
- 173. Brooks.—See No. 48 this list.
- 174. Bumpus.—See No. 49 this list.
- 175. Claus and Sedgwick.—See No. 18 this list, vol. i.

- 176. Gegenbaur.—See No. 19 this list.
- 177. Griffiths.—See No. 53 this list.
- 178. Huxley.—See No. 21 this list.
- 179. Hyatt.—See No. 155 this list.
- 180. Parker.—See No. 44 this list.
- 181. Romanes.—“Jelly-fish, Starfish, and Sea-urchin,” in “International Scientific Series,” New York, 1885.
- 182. “Standard Natural History.”—See No. 26 this list, vol. i.

## EARTHWORM

- 183. Brooks.—See No. 48 this list.
- 184. Claus and Sedgwick.—See No. 18 this list, vol. i.
- 185. Darwin.—“Formation of Vegetable Mould through the Action of Earthworms,” New York, 1881.
- 186. Davis.—See No. 51 this list.
- 187. Gegenbaur.—See No. 19 this list.
- 188. Griffiths.—See No. 53 this list.
- 189. Howes.—See No. 9 this list.
- 190. Huxley and Martin.—See No. 10 this list.
- 191. Hyatt.—“Worms and Crustacea,” in “Guides for Science Teaching,” No. VII., Boston, 1885.
- 192. Lang.—See No. 22 this list.
- 193. Marshall and Hurst.—See No. 12 this list.
- 193a. Morgan.—See No. 22a this list.
- 194. Packard.—See No. 24 this list.
- 195. Sedgwick and Wilson.—“General Biology,” Part I., New York, 1886.
- 196. “Standard Natural History.”—See No. 26 this list, vol. i.

## LOBSTER

- 197. Brooks.—See No. 48 this list.
- 198. Bumpus.—See No. 49 this list.
- 199. Claus and Sedgwick.—See No. 18 this list, vol. i.
- 200. Davis.—See No. 51 this list.
- 201. Gegenbaur.—See No. 19 this list.
- 202. Griffiths.—See No. 53 this list.

- 203. Howes.—See No. 9 this list.
- 204. Huxley. — “The Crayfish,” in “International Scientific Series,” New York, 1880.
- 205. Huxley.—See No. 21 this list.
- 206. Huxley and Martin.—See No. 10 this list.
- 207. Hyatt.—See No. 191 this list.
- 208. Lang.—See No. 22 this list.
- 209. Marshall and Hurst.—See No. 12 this list.
- 209a. Morgan.—See No. 22a this list.
- 210. Packard.—See No. 24 this list.
- 211. Parker.—See No. 44 this list.
- 212. “Standard Natural History.”—See No. 26 this list, vol. ii.
- 213. Woodward.—Article “Crustacea,” in “Encyclopædia Britannica,” Ninth edition.

## LOCUST

- 214. Brooks.—See No. 48 this list.
- 215. Bumpus.—See No. 49 this list.
- 216. Claus and Sedgwick.—See No. 18 this list, vol. i.
- 217. Comstock.—“Introduction to Entomology,” Ithaca, 1888.
- 218. Gegenbaur.—See No. 19 this list.
- 219. Griffiths.—See No. 53 this list.
- 220. Huxley.—See No. 21 this list.
- 221. Hyatt and Arms. — “Insecta,” in “Guides for Science Teaching,” No. VIII., Boston, 1890.
- 222. Lang.—See No. 22 this list.
- 223. Miall and Denny.—“The Cockroach,” London, 1886.
- 224. Packard.—“Guide to the Study of Insects,” New York, 1889.
- 225. Packard.—See No. 24 this list.
- 226. “Reports U. S. Entomological Commission for 1877,” Washington, 1878.
- 227. “Reports U. S. Entomological Commission for 1878 and 1879,” Washington, 1883.
- 228. “Reports U. S. Entomological Commission,” Washington, 1883.
- 229. “Standard Natural History.”—See No. 26 this list, vol. ii.

## MOLLUSCO

- 230. Brooks.—"The Oyster," Baltimore, 1891.
- 231. Brooks.—See No. 48 this list.
- 232. Claus and Sedgwick.—See No. 18 this list, vol. ii.
- 233. Davis.—See No. 51 this list.
- 234. Gegenbaur.—See No. 19 this list.
- 235. Griffiths.—See No. 53 this list.
- 236. Howes.—See No. 9 this list.
- 237. Huxley.—See No. 21 this list.
- 238. Huxley and Martin.—See No. 10 this list.
- 239. Hyatt.—"Oyster, Clam, and other Common Mollusca,"  
in "Guides for Science Teaching," No. VI., Boston,  
1884.
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- 264. Parker.—See No. 44 this list.
- 265. Sachs.—See No. 34 this list.
- 266. Strasburger.—See No. 15 this list.
- 267. Wolle.—See No. 137 this list.

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- 270. Davis.—See No. 51 this list.
- 271. Goebel.—See No. 31 this list.
- 272. Howes.—See No. 9 this list.
- 273. Huxley and Martin.—See No. 10 this list.
- 274. Parker.—See No. 44 this list.
- 275. Sachs.—See No. 34 this list.
- 276. Strasburger.—See No. 15 this list.

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- 278. Bower and Vines.—See No. 3 this list.
- 279. Campbell.—See No. 30 this list.



- 280. Davis.—See No. 51 this list.
- 281. Goebel.—See No. 31 this list.
- 282. Sachs.—See No. 34 this list.
- 283. Strasburger.—See No. 15 this list.

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- 284. Campbell.—See No. 30 this list.
- 285. Cooke and Berkeley.—See No. 119 this list.
- 286. Davis.—See No. 51 this list.
- 287. De Bary.—See No. 103 this list.
- 288. Goebel.—See No. 31 this list.
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- 290. Huxley and Martin.—See No. 10 this list.
- 291. Parker.—See No. 44 this list.
- 292. Strasburger.—See No. 15 this list.

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- 293. Bower and Vines.—See No. 3 this list.
- 294. Campbell.—See No. 30 this list.
- 295. De Bary.—See No. 103 this list.
- 296. Goebel.—See No. 31 this list.
- 297. Parker.—See No. 44 this list.
- 298. Sachs.—See No. 34 this list.
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## MARCHANTIA

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- 301. Bower and Vines.—See No. 3 this list.
- 302. Goebel.—See No. 31 this list.
- 303. Sachs.—See No. 34 this list.
- 304. Strasburger.—See No. 15 this list.

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## INDEX AND GLOSSARY

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[In preparing the glossary it has been thought advisable to omit the Greek and Latin words from which the various biological terms are derived. This has been done partly to save space and partly for the reason that most students in scientific courses are not familiar with the classical languages, especially with the Greek. The English equivalents of such words are given, and in most cases define the term with sufficient accuracy.]

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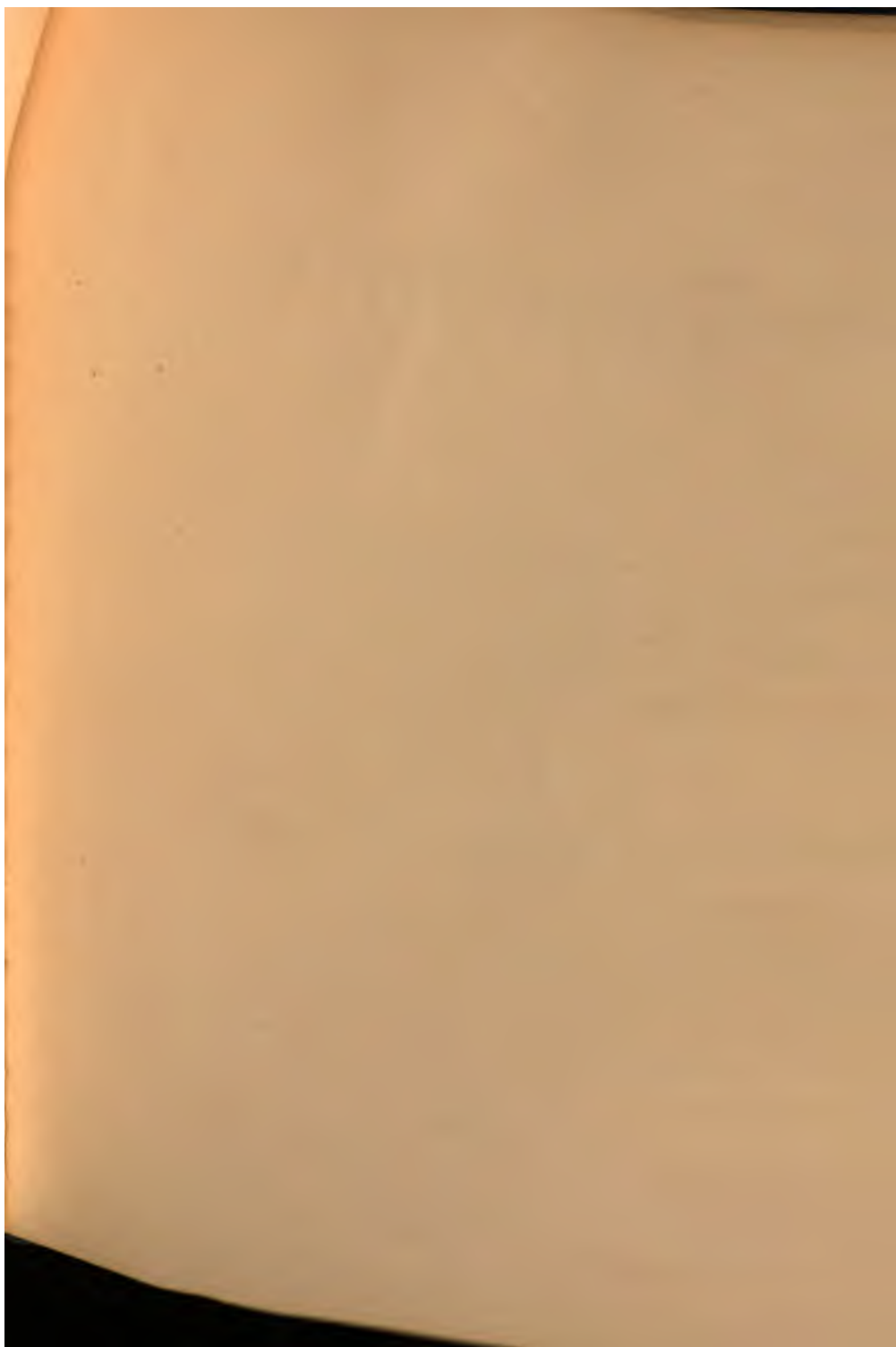
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